

A KARYOTYPE ANALYSIS OF THE GENUS *XENOPUS* (*ANURA, PIPIDAE*)

THÈSE

Présentée à la faculté des Sciences
de l'Université de Genève
pour obtenir le grade de docteur ès sciences biologiques

par

Janina TYMOWSKA

de

Pologne

Thèse n° 1781

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La faculté des sciences, sur le préavis de Messieurs M. FISCHBERG, professeur ordinaire et directeur de thèse, R. MATTHEY, professeur (Université de Lausanne) et Madame M. NARBEL, docteur ès sciences (Lausanne) autorise l'impression de la présente thèse, sans exprimer d'opinion sur les propositions qui y sont énoncées.

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Le Doyen
Jean MULLER

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A

MES PARENTS

ET

MES ENFANTS

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GENERAL INTRODUCTION

In the course of the last twenty years the clawed toad X.laevis has become an important animal in various fields of biological research. In particular, the finding of a mutation affecting the nucleolar number (ELSDALE et al. 1958, FISCHBERG and WALLACE, 1960) initiated studies on the developmental genetics of this organism which provided an insight into the phenomenon of gene regulation and the control of rRNA synthesis. Owing to the popularity of Xenopus in laboratory studies, a detailed cytological analysis of this genus appeared necessary and has been undertaken.

The genus Xenopus belongs to one of the oldest families of Anura, the Pipidae and represents one of the three genera of the sub-family Xenopinae living in Africa. It is considered the most primitive of the three genera within this sub-family (NOBLE, 1954). Within this genus six species have until recently been distinguished : X.laevis (Daudin), X.gilli Rose et Hewitt, X.muelleri (Peters), X.clivii Peracca, X.tropicalis (Gray), X.fraseri Boulenger and in the species X.laevis seven subspecies have been described : X.l.laevis (Daudin), X.l.victorianus Ahl, X.l.petersi Bocage, X.l.borealis Parker, X.l.bunyoniensis Loveridge, X.l.poweri Hewitt and X.l.sudanensis Perret (NIEUWKOOP and FABER, 1967).

The systematics of the genus Xenopus has, however, recently been modified, mainly by research carried out in our laboratory. Blackler and Fischberg (1968) doubt if X.l.poweri is different from X.l.petersi and the merit of its subspecific status is, therefore, contested. X.l.bunyoniensis is only known from museum collections, repeated efforts to find live specimens in all its previous habitats have not been successful. It might now be extinct. The particular morphology of museum specimens suggests that it might merit the status of a species (TINSLEY, 1975). Xenopus l. borealis has been removed from the "Rassenkreis" laevis and been elevated to a species X.borealis. If crossed with X.l.laevis hybrid sterility results (FISCHBERG, personal communication). Hybrid females show mainly univalents in their oocytes (MOLLER, unpublished). As can be seen later, the karyological analysis also supports this view.

Finally three new species have been found recently and have now to be added to the six previously known ones. Xenopus vestitus Laurent (LAURENT 1972), which is synonymous with X.kigesiensis Tinsley, described independently and almost simultaneously by R.C. Tinsley (1973, 1975). Xenopus ruwenzoriensis Fischberg and Kobel (in manuscript, see also TYMOWSKA and FISCHBERG 1973) is another new species from Western Uganda like the so far unnamed species X.sp.n. Fischberg, Tinsley and Kobel (in preparation).

Previous cytological work on this genus is almost entirely limited to X.laevis laevis. Its chromosome number has been determined by Wickbom (1945) as $2n = 36$. The female heterogamety of this species has been established by experiments on sex reversal (ZW) (GALLIEN, 1955, 1956; CHANG and WITSCHI, 1955, 1956; BLACKLER, 1965). Further studies on the X.laevis karyotype provoked disagreement as to the existence of heteromorphism of sex-chromosomes. Weiler and Ohno (1962) and Morescalchi (1963) have claimed that the sex-chromosomes of Xenopus laevis are heteromorphic, thereby making Xenopus an exception amongst amphibians. According to their studies Xenopus females possess a large submetacentric chromosome W and a small acrocentric chromosome Z. Karyological analysis of normal and sex reversed Xenopus laevis females carried out by Mikamo and Witschi (1966) have not shown heteromorphic pairs of chromosomes. By using autoradiographic methods, Gehring and Hauschtek-Jungen were not able to recognize the sex-chromosomes due to any lack of synchrony during mitotic metaphase.

The discovery of the Oxford nucleolar mutation (ELSDALE et al., 1958; FISCHBERG and WALLACE, 1960) revealed another interesting feature of the Xenopus laevis karyotype. Kahn (1962) reported the absence of one secondary constriction in the chromosome complement of X.l.laevis heterozygous for the nucleolar mutation. He concluded, therefore, that the nucleolar mutation was due to the deletion of the nucleolar organizer region which corresponds to the secondary

constriction on the satellite chromosome. Rafferty and Sherwin (1969) measured this constriction and found that the length of the nucleolar constriction "depends on the amount of genome" and "that the deletion is limited to the constricted segment".

The only cytological work carried out with another Xenopus species concerns "X.mulleri". But due to the fact that most scientists, working with "mulleri" imported into America, mistook X.borealis for X.muelleri, some confusion exists as to their results. Pardue (1974) hybridised rRNA with the terminal heterochromatin and proved this constriction to be the nucleolar organizer. Morescalchi (1973) confirmed the chromosomal number of X.muelleri as $2n = 36$.

In this study the chromosome number and the karyotypes of all as yet known Xenopus species and subspecies have been established with the only exception of X.l.bunyoniensis, X.l.poweri and X.l.sudanensis. These three X.laevis subspecies were not available. As will be seen later the chromosome numbers of X.tropicalis, X.vestitus, X.sp.n. and X.ruwenzoriensis proved to be particularly interesting and rather exceptional for amphibians. In addition the karyotypes of the different species have been compared with one another and particular attention has been given to the nucleolar organizer region and the problem of sex-chromosome heteromorphism.

Karyotype analysis of *Xenopus muelleri* (PETERS) and *Xenopus laevis* (DAUDIN), Pipidae¹

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Abstract

A cytogenetic analysis of two anuran species, *Xenopus laevis laevis* and *Xenopus muelleri*, was performed. The diploid number of chromosomes in both species was $2n = 36$. The two karyotypes, arranged according to size of chromosome and centromere position, showed no pronounced differences. However, the chromosomes which had a secondary constriction were different. In *X. laevis* this constriction (nucleolar organizer) was found on one pair of acrocentric chromosomes only, the pair showing association in about 50% of the metaphases. In *X. muelleri*, on the other hand, two distinct pairs of submetacentric chromosomes contained secondary constrictions. Neither of these two chromosome pairs was morphologically similar to the constriction-bearing chromosomes of *X. laevis*. Association similar to that in *X. laevis* was found in one pair only, the one with a terminal constriction. In neither of the two species was there any evidence of sex-chromosome heteromorphism.

WICKBOM (1945) determined the chromosome number of *Xenopus laevis* as $2n = 36$. WEILER and OHNO (1962) claimed that the sex-determining chromosomes of this anuran are heteromorphic, thereby singling out *X. laevis* as the only amphibian known to manifest this chromosomal heteromorphism. According to WEILER and OHNO, the W-chromosome (in *Xenopus*, the female is the heterogametic sex, WZ [GALLIEN, 1955, 1956; CHANG and WITSCHI, 1955, 1956]) is the largest submetacentric chromosome, whereas the Z-chromosome is the smallest acrocentric one. However, in a later study MIKAMO and WITSCHI (1966) denied the validity of the previous claim for the existence of chromosomal heteromorphism. Moreover, radioactive

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isotope incorporation indicated a synchronous replication of all the chromosomes (GEHRING and HAUSCHTECK JUNGES, 1967).

The karyotype of *X. laevis* became of particular interest because of the presence of a chromosome pair which carries a secondary constriction, associated with the formation of nucleoli (KAHN, 1962). The presence or absence of the constriction (nucleolar organizer) is in accordance with the behavior of the nucleolar mutation described by ELSDALE et al. (1958) and FISCHBERG and WALLACE (1960). RAFFERTY and SHERWIN (1969) published measurements of this secondary constriction in *X. laevis*.

Our present concern with the karyotype arises from the fact that *X. laevis* forms viable hybrids with another *Xenopus* species, *X. muelleri*. This hybrid presents unusual features of nucleolus formation during early development (KOBEL and FISCHBERG, unpublished). The necessity to examine the chromosomes of *X. muelleri* gave us the opportunity to compare morphometrically the karyotypes of both species.

Materials and methods

The *X. laevis* used were imported from Fish Hoek, Cape, South Africa, whereas the *X. muelleri* originated from Jfakara, Tanzania.

Chromosome preparations were made of adult animals, using two males and two females of each species. Animals were injected intraperitoneally with 25 IU of chorionic gonadotropin (Antex, Leo) three days prior to the injection of 0.5 ml of 0.1% Colchicin (Merck). Five hours later, chromosome preparations were made of the spleen and testis using a modified air-drying method (EVANS et al., 1964; MEREDITH, 1969).

For the karyotype analysis of each animal, we examined about 10 mitotic metaphase plates in which the chromosomes were neither overlapping nor excessively contracted. The quantitative characterization required measurements of the relative length (expressed as per mille of the total length of the diploid chromosomal set) and the arm ratio (index: short arm/long arm $\times 100$). These data served for the construction of the idiograms and of the two-dimensional karyograms (KOBEL, 1967).

Results and discussion

For both *X. muelleri* and *X. laevis*, the diploid chromosome number is $2n = 36$. The karyotypes of the two species are morphologically similar, with the exception of the chromosomes bearing secondary constrictions (figs. 1 and 2).

Measurements of the relative length and the arm ratio for each chromo-



Fig. 1. Mitotic chromosomes and karyotype of *Xenopus laevis*. Arrows indicate the chromosomes bearing secondary constrictions.

TYMOWSKA, KOBEL Karyotype of *X. muelleri* and *X. laevis*



Fig. 2. Mitotic chromosomes and karyotype of *Xenopus muelleri*. Arrows indicate the chromosomes bearing secondary constrictions.

TYMOWSKA, KOBEL Karyotype of *X. muelleri* and *X. laevis*Table I. Morphometric characteristics of the chromosomes of *Xenopus laevis* and *Xenopus muelleri*.

Pair No.	Relative length (% of 2n set)			Arm ratio ($\frac{\text{short}}{\text{long}} \times 100$)		
	$\bar{x}$	$\pm s$	$\pm s_{\bar{x}}$	$\bar{x}$	$\pm s$	$\pm s_{\bar{x}}$
<i>Xenopus laevis</i> (2♂, 2♀; 37 mitoses)						
1	40.35	2.92	0.34	70.57	8.17	0.95
2	36.55	3.50	0.41	62.38	6.55	0.75
3	33.56	2.16	0.25	59.47	6.16	0.72
4	29.98	1.49	0.17	60.33	7.42	0.86
5	26.97	1.82	0.21	61.70	9.28	1.86
6	25.36	1.38	0.16	67.17	7.64	0.89
7	24.42	1.80	0.21	67.95	11.64	1.30
8	30.34	1.99	0.23	49.27	6.75	0.78
9	29.09	1.66	0.19	89.77	7.16	0.83
10	21.05	2.00	0.53	84.08	11.32	1.32
11	19.78	1.59	0.18	87.94	9.85	1.14
12	33.34	3.55	0.41	35.49	9.79	1.14
13	28.08	1.76	0.20	34.00	7.20	0.84
14	26.44	2.26	0.26	29.85	7.18	0.83
15	25.24	1.58	0.18	28.42	5.85	0.68
16	24.67	1.63	0.19	29.09	6.09	0.71
17	23.71	1.59	0.18	31.51	6.76	0.76
18	21.95	1.54	0.18	35.08	7.18	0.83
<i>Xenopus muelleri</i> (2♂, 2♀; 38 mitoses)						
1	40.94	3.15	0.36	69.08	6.53	0.74
2	35.76	2.46	0.28	62.20	7.21	0.83
3	33.10	2.26	0.26	58.96	6.32	0.72
4	34.50	3.17	0.36	66.82	11.14	1.28
5	29.65	2.15	0.24	59.83	6.94	0.79
6	25.57	1.80	0.20	73.37	7.22	0.83
7	32.38	3.08	0.35	74.32	10.13	1.16
8	27.49	1.89	0.22	37.96	6.01	0.69
9	28.49	2.13	0.24	88.32	8.19	0.94
10	23.47	1.93	0.22	75.07	9.11	1.05
11	21.20	1.63	0.19	75.72	10.80	1.24
12	26.90	2.00	0.23	22.57	4.62	0.53
13	24.70	1.70	0.19	33.85	7.65	0.88
14	24.42	1.99	0.23	29.64	6.49	0.74
15	23.75	2.10	0.24	30.99	6.75	0.77
16	23.04	1.81	0.21	32.28	7.15	0.82
17	21.55	1.69	0.19	30.58	7.17	0.88
18	20.59	2.05	0.24	30.03	5.50	0.63

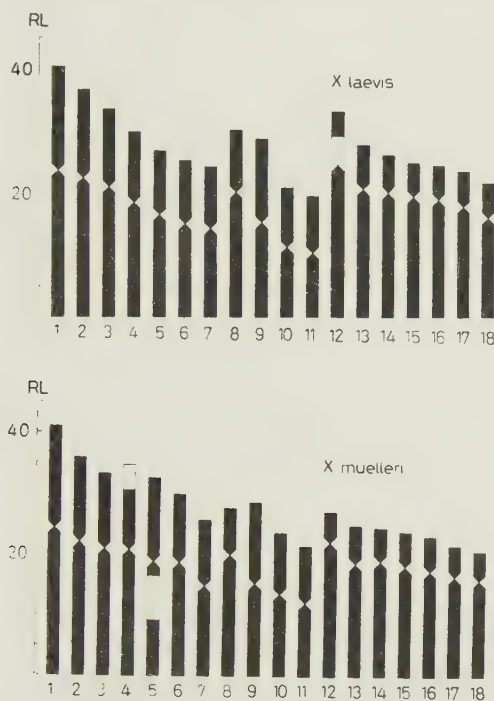
TYMOWSKA, KOBEL Karyotype of *X. muelleri* and *X. laevis*

Fig. 3. Idiograms of *Xenopus laevis* and *Xenopus muelleri* based on the measurements of relative length and arm ratio.

some permitted a detailed classification. The results are presented in table I and figs. 3 and 4.

The chromosomes of *X. laevis* fall into seven groups (fig. 1). The three longest submetacentric pairs (Nos. 1 to 3) form one group. The longest pair (No. 1) is characterized by the difference between the homologs, one of which is always longer than the other; thus we concur with the observation of MIKAMO and WITSCHI (1966). The second group is composed of four pairs of submetacentric chromosomes (Nos. 4 to 7) which are all very much alike. The third group contains a single pair (No. 8). The next group is also made up of a single pair of long metacentric chromosomes (No. 9). The last two metacentric pairs (Nos. 10 and 11) are the smallest ones and form their own group. The longest of the seven acrocentric pairs is characterized by a 'secondary constriction on the short arms and forms, therefore, a separate group of satellited chromosomes (pair No. 12). The remaining six pairs (Nos. 13 to 18) belong to a single group, as no distinguishing features can be observed.

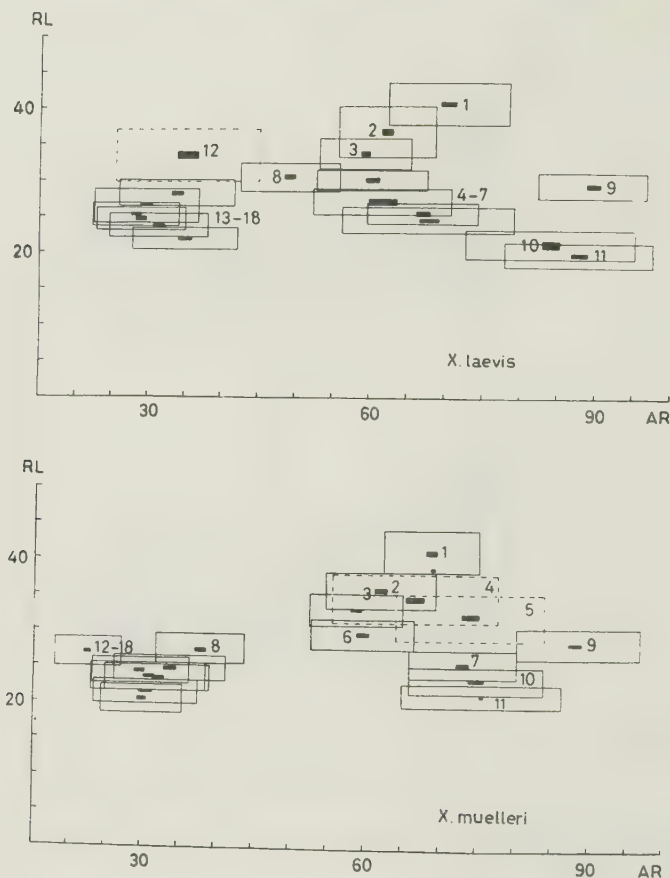


Fig. 4. Two-dimensional karyograms of *Xenopus laevis* and *Xenopus muelleri*. Black squares indicate the error of the mean and white squares the standard deviation of the relative length (RL, ordinate) and of the arm ratio (AR). Chromosomes carrying secondary constrictions are designated by dotted lines.

The chromosomes of *X. muelleri* fall into eight groups (fig. 2). The first group is composed of the three longest submetacentric pairs (Nos. 1 to 3), as in *X. laevis*. The next four pairs of shorter submetacentric chromosomes (Nos. 4 to 7) belong to three groups. There are two pairs of chromosomes (Nos. 4 and 5) that show basic differences from the *laevis* chromosomes; both bear secondary constrictions. In pair No. 4 (fig. 2), the constriction is placed terminally as a prolongation of the short arm. In the other pair, No. 5 (fig. 2), the constriction appears within the long arm, separated from the

centromere by a short condensed region and from the end of the arm by a long satellite. Each of these pairs forms its own group. The remaining group contains the similar pairs Nos. 6 and 7. Pairs Nos. 8 to 11 are classified as in *laevis* (three groups), but all acrocentric pairs of *muelleri* (Nos. 12 to 18) form a single group, since significant morphological differences have not been observed.

In *X. laevis* the secondary constrictions of the satellite chromosomes are considered to be nucleolar organizers (KAHN, 1962; TYMOWSKA and FISCHBERG, in preparation.) Since diploid nuclei of *X. muelleri* do not possess more than two nucleoli, it is probable that the latter are formed by only one of the two chromosome pairs which bear secondary constrictions. It could be either No. 4 or No. 5. However, observations on *laevis* mitotic metaphase plates show that the two homologous chromosomes possessing nucleolar organizers (No. 12) show chromosome association in about 50% of the plates (table II). This could be interpreted as the consequence of their prior attachment to a single fused nucleolus (WALLACE, 1963). In *X. muelleri* such an association of the two homologs is observed with similar frequency for pair No. 4 exclusively (table II). The chromosomes of pair No. 5 do not show such behavior. It would be interesting to demonstrate with certainty which of the secondary constrictions of the two different chromosome pairs, if not both of them, play the role of nucleolar organizers in *X. muelleri*.

Our examination of the karyotypes of both sexes in both species failed to reveal any morphological variance between the members of any one pair of chromosomes, such as might be expected for a WZ-pair. Since the difference in relative length between the homologs of the longest chromosome pair (No. 1) occurs in either sex of *X. laevis*, we cannot, therefore, support the claim of WEILER and OHNO (1962).

Table II. Frequency of association of homologous chromosomes, bearing secondary constrictions, in metaphase plates.

	Homologs associated	Homologs separated	Total of metaphases
<i>Xenopus laevis</i>			
Pair No. 12	19	18	37
<i>Xenopus muelleri</i>			
Pair No. 4	19	19	38
Pair No. 5	0	38	38

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Karyotype analysis of *Xenopus tropicalis* GRAY, Pipidae¹

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Abstract

A cytological analysis of the anuran species *Xenopus tropicalis* was performed. The diploid chromosome number is $2n = 20$, whereas two other *Xenopus* species previously analyzed, *laevis* and *muelleri*, have $2n = 36$. In *X. tropicalis* the morphology of the various chromosome groups differs from the other two species. The karyotype arranged according to size of chromosomes and centromere position falls into six morphological groups of chromosomes. Two distinct pairs bear secondary constrictions. Neither of these is similar to the constriction-bearing chromosomes of *X. laevis* (nucleolar organizer) or *X. muelleri*. The aspect of their association is also different. There is no evidence for sex-chromosome heteromorphism. The karyotype analysis suggests that *X. tropicalis* is systematically quite separate from the two other species mentioned above.

A recent comparison of the karyotypes of the two anuran species *X. laevis laevis* and *X. muelleri* (TYMOWSKA and KOBEL, 1972) showed a diploid chromosome number for both species of $2n = 36$. There is no fundamental difference between the two karyotypes, but the chromosomes bearing secondary constrictions are different. In *X. laevis* the secondary constrictions (considered to be nucleolar organizers [KHAN, 1962; RAFFERTY and SMERVIN, 1969]) appear in one pair of chromosomes only. In *X. muelleri* two distinct pairs of chromosomes possess secondary constrictions (TYMOWSKA and KOBEL, 1972). There is no absolute evidence as to which of these two pairs present the nucleolar organizers. However, on the basis of chromosome association, one can observe an interesting

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TYMOWSKA Karyotype of *Xenopus tropicalis*

analogy between the two species. In *X. laevis* the two homologous chromosomes with the nucleolar organizer are associated in 50 % of the metaphases. In *X. muelleri* a similar behavior was observed exclusively for the pair possessing the terminal secondary constrictions.

In both *X. laevis* and *X. muelleri* a comparison of the karyotypes of the two sexes failed to show any chromosomal sex dimorphism (MIKAMO and WITSCHI, 1966; GEHRING and HAUSCHTECK JUNGES, 1967; TYMOWSKA and KOBEL, 1972). This observation, therefore, does not support the claim of WEILER and OHNO (1962) and MORESCALCHI (1963) that the sex-determining chromosomes of *X. laevis* are heteromorphic.

In this paper another *Xenopus* species, *X. tropicalis*, has been cytologically analyzed, not only in order to establish the karyotype but also in respect to chromosomal association and possible chromosomal heteromorphism.

Materials and methods

Our *X. tropicalis* were imported from Adiopodoumé, Côte d'Ivoire. Four adult animals, two males and two females, were used for the chromosomal analyses. The chromosome preparations and the karyotype examination of 10 mitotic plates per animal were made by the method previously described (TYMOWSKA and KOBEL, 1972).

Results and discussion

The diploid number of *Xenopus tropicalis* is $2n = 20$. During male meiosis 10 bivalents were seen in metaphase I and 10 chromosomes in metaphase II (fig. 4c, d). During mitotic metaphases 20 chromosomes were regularly observed (fig. 1). The other known *Xenopus* species, *X. laevis* (WICKBOM, 1954) and *X. muelleri* (TYMOWSKA and KOBEL, 1972), have $2n = 36$.

Measurements of the relative length and the arm ratio for each chromosome of *X. tropicalis* made a detailed classification possible. The results are presented in table I and in figs. 1 to 3.

The chromosomes fall into six groups (figs. 1 and 3). The four longest submetacentric chromosomes (Nos. 1 to 4) compose the first group. However, pair No. 1 can easily be distinguished from the others. The next two groups are each formed by a single pair of chromosomes which

TYMOWSKA Karyotype of *Xenopus tropicalis*

Fig. 1. Mitotic chromosomes and karyotype of *X. tropicalis*. Note the secondary constrictions indicated by arrows.

bear secondary constrictions (Nos. 5 and 6). The constrictions appear within the long arms, separated from the centromeres and the ends of the arms by condensed regions. Pair No. 5 is larger than pair No. 6. Apart from this, the frequency in the appearance of a secondary constriction varies. In pair No. 5 it can be observed in 96 % of the metaphases, whereas in pair No. 6 it is found in 36 % only. The fourth group is made up of the smallest pair of chromosomes, No. 7, which is also submetacentric. The long metacentric chromosome pair, No. 8, forms its own group. The last group is composed of two pairs of acrocentric chromosomes (Nos. 9 and 10) which can be distinguished from each other.

The karyotype of *X. tropicalis* is very different from the karyotypes of *X. laevis* and *X. muelleri*. The chromosomes of *tropicalis* form different morphological groups which cannot be compared directly with the groups

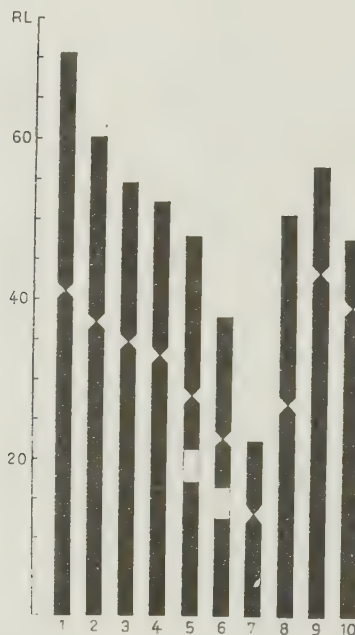
TYMOWSKA Karyotype of *Xenopus tropicalis*

Fig. 2. Idiogram of *X. tropicalis* based on the measurements of relative length and arm ratio.

of *laevis* and *muelleri*. Both chromosome pairs which bear secondary constrictions differ not only morphologically but also in their association behavior from those of the other two species. Observation of mitotic *tropicalis* metaphase plates shows that the two homologous chromosomes of pairs Nos. 5 and 6 lie next to each other in 30 % and 17 %, respectively, of the metaphases examined (fig. 4a, b). In *laevis* and *muelleri*, on the other hand, associated chromosomes show real physical attachment. The different behavior of the homologous chromosomes bearing secondary constrictions in *X. tropicalis* could possibly be explained by the morphological differences between the analogous pairs in *laevis* and *muelleri*. In *X. laevis* the secondary constriction appears as a short arm with a satellite; in *X. muelleri* the secondary constrictions of the associated chromosomes are terminal. Therefore, in both species attachment might be easier than for *tropicalis*, where the secondary constrictions appear between long regions of condensed chromatin.

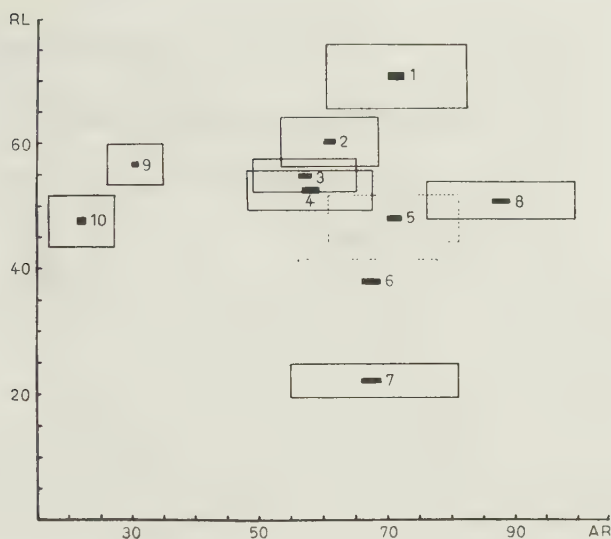
TYMOWSKA Karyotype of *Xenopus tropicalis*

Fig. 3. Two-dimensional karyogram of *X. tropicalis*. Black squares indicate the standard error of the mean and white squares the standard deviation of the relative length (RL, ordinate) and of the arm ratio (AR). Chromosomes carrying secondary constrictions are designated by dotted lines.

Table I. Morphometric characteristics of the chromosomes of *Xenopus tropicalis* (2♀♀, 2♂♂; 40 mitoses).

Pair No.	Relative length (% of 2n set)			Arm ratio ($\frac{\text{short}}{\text{long}} \times 100$)		
	Mean	SD	SE	Mean	SD	SE
1	70.59	5.07	0.57	71.81	11.11	1.24
2	60.05	3.93	0.44	61.25	7.71	0.86
3	54.62	2.59	0.29	57.38	8.10	0.90
4	52.25	3.18	0.35	58.33	9.85	1.10
5	50.62	2.99	0.33	87.96	11.46	1.28
6	56.61	3.28	0.37	30.59	4.53	0.51
7	47.58	4.04	0.45	22.09	5.15	0.58
8	47.77	3.67	0.41	71.27	10.03	1.12
9	37.84	3.45	0.38	67.58	12.26	1.37
10	22.18	2.69	0.30	67.52	13.61	1.52

TYMOWSKA Karyotype of *Xenopus tropicalis*

Fig. 4. a, b. Two mitotic metaphases of *X. tropicalis* showing the close proximity of chromosomes bearing secondary constrictions (arrows). c. Metaphase of first spermatogonial division showing 10 bivalents. d. Metaphase of second spermatogonial division showing 10 chromosomes.

Since diploid nuclei of *X. tropicalis* show at most only two nucleoli, the secondary constriction of chromosome pair No. 5 or No. 6 might represent the nucleolar organizer region. Since the constriction of chromosome pair No. 5 is visible in 96 % of the metaphase plates, it is probably the nucleolar organizer. The close proximity of the homologs in 30 % of the analyzed mitoses could be interpreted as the consequence of their prior attachment to a single fused nucleolus (WALLACE, 1963).

The 20 chromosomes of *X. tropicalis* represent 55.6 % of the 36 chromosomes of *X. laevis* and *X. muelleri*. Measurements made of the total length of the diploid complements for all three species are presented in an idiogram (fig. 5); it shows that the *tropicalis* chromosomes are not longer than those of the other two species. The diploid genome length of *X. tropicalis* is 52.1 % of that of *X. laevis* and 49.4 % of that of *X. muelleri*. OLMOE et al. (1970) found by histophotometric methods a nucleolar DNA

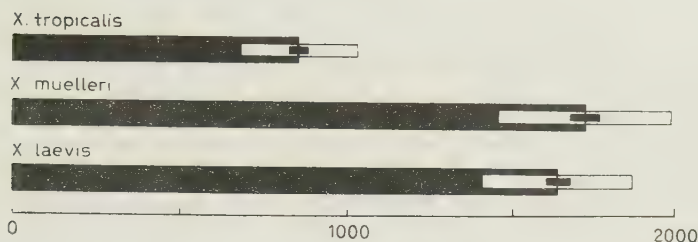
TYMOWSKA Karyotype of *Xenopus tropicalis*

Fig. 5. Total length of chromosome complements of *X. tropicalis* (mean = 855.15), *X. muelleri* (mean = 1729.65), and *X. laevis* (mean = 1601.83). White squares indicate the standard deviation and black squares the standard error of the mean.

content of 6.2 pg/N for *laevis* and 8.2 pg/N for *muelleri*. The ratio of these data (100:132.25) differs from our ratio of diploid genome length between *X. laevis* and *X. muelleri* (100:105.4), but the tendency is the same. The length of the *X. tropicalis* chromosome complement is approximately half that of the other two species. One can therefore assume that the nuclear DNA content of *X. tropicalis* is considerably smaller. It is interesting to see that, in anuran amphibians, species of similar complexity can vary so greatly in the length of their chromosome complements.

The karyotype analysis of *X. tropicalis* did not show any morphological variance of sex chromosomes in either of the two sexes.

X. tropicalis shows important differences in chromosome number, total length of chromosome complement, and chromosome morphology from *X. laevis* and *X. muelleri*; therefore it can be concluded that this particular species is systematically quite distant from the two species previously analyzed (TYMOWSKA and KOBEL, 1972).

Acknowledgements

The author wishes to thank Prof. M. FISCHBERG and Dr. H. KOBEL for their interest and support of this work and Miss H. ACHERMANN and Mr. P. GRUTTER for their skilful technical help.

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Running head

TYMOWSKA, Comparative study of Xenopus karyotypes

Title

A comparative study of the karyotypes of eight Xenopus species and subspecies which possess a 36-chromosome complement ¹⁾.

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ABSTRACT

A comparative study of the karyotypes of eight Xenopus species and subspecies possessing a diploid number of 36 chromosomes (X.l.laevis, X.l.petersi, X.l.victorianus, X.borealis, X.gilli, X.fraseri, X.clivii) was performed. Uniform karyotypes based on the morphology and size of chromosomes differ only by the type and position of secondary constrictions. Some of these constrictions are nucleolar organizers, which can be recognized owing to the characteristic behavior of mitotic chromosomal association. On the basis of a comparison of the chromosomes bearing secondary constrictions, an attempt is made to establish the cytotaxonomic relationships. There is confirmation of the close relationship among the subspecies X.l.laevis, X.l.petersi, X.l.victorianus in contrast to X.borealis which is a separate species much nearer to X.muelleri. The karyotype of X.gilli is interpreted as being intermediate to these two groups. X.clivii could possibly be regarded as a species close to the group of X.muelleri. X.fraseri, having a particular type of secondary constriction, is systematically more distant.

INTRODUCTION

Due to the popularity of Xenopus for research in various fields of biology, a cytogenetic study of this genus appeared necessary and has been undertaken.

The genus Xenopus belongs to one of the oldest families of Anura, the Pipidae, and represents one of the three genera of the sub-family Xenopinae living in Africa. It is considered the most primitive of the three genera within this sub-family (NOBLE, 1954). The karyotype of Xenopus is unique among the present Anura, and there are difficulties to find any morphological connection between the karyotype of Xenopus and other Pipids (MORESCALCHI, 1968a; 1973).

The basic chromosome number of the genus Xenopus is $x = 18$. The majority of species possess 36 chromosomes, however, there are also natural polyploid species like the tetraploid species X. vestitus (TYMOWSKA and TINSLEY, in preparation) ($4n = 72$) and the hexaploid species of X. ruwenzoriensis ($6n = 108$), (TYMOWSKA and FISCHBERG, 1973). X. tropicalis presents a separate case in which the diploid number is 20 (TYMOWSKA, 1973).

The present cytological analysis deals only with most of those Xenopus species which possess 36 chromosomes. The following species and subspecies have been examined : X. laevis laevis (Daudin), X. laevis petersi Bocage, X. laevis victorianus Ahl, X. borealis (Parker), X. muelleri (Peters), X. gilli Rose et Hewitt, X. clivii Peracca and X. fraseri Boulenger.

Attempts have been made to establish phylogenetic relationships between these species by means of detailed karyotype comparison.

MATERIALS AND METHODS

The animals originated from the following localities :

<u>Xenopus laevis laevis</u>	from Fish Hoek, Cape, South Africa
<u>Xenopus laevis petersi</u>	from Northern Rhodesia
<u>Xenopus laevis victorianus</u>	from Kampala, Uganda
<u>Xenopus borealis</u>	from Nairobi, Kenya
<u>Xenopus muelleri</u>	from Ifakara, Tanzania
<u>Xenopus gilli</u>	from Cape, South Africa
<u>Xenopus clivii</u>	from Ethiopia
<u>Xenopus fraseri</u>	from Foulassi, Cameroun

Four adult animals of each species, two females and two males, were examined. The only exception to this was X.gilli where it was only possible to obtain two animals for examination. The direct chromosomal preparations were made by the dry-air method described previously (TYMOWSKA and KOBEL, 1972). The karyotype analyses were based on 10 karyotypes of each animal. The chromosomes were measured and arranged into groups according to their size and centromere positions.

The classification of arm ratios proposed by Levan et al. (1964) was applied, although we used those terms most commonly found in the literatures (i.e. metacentric, submetacentric, subacrocentric, acrocentric and telocentric).

The measurements of chromosomes permitted the calculation of the total absolute length of diploid complements as well as the relative lengths of single chromosomes

(RL = per mille of the total length of the diploid set) and their arm ratios ($AR = \text{short arm} / \text{long arm} \times 100$). These quantitative characterizations are represented in Tables I, II, III and V, idiograms (figs. 1, 2, 6, 9) and two-dimensional karyograms (fig. 7).

RESULTS

Number of chromosomes

The chromosomal number for all subspecies and species presented in this paper is $2n = 36$, (WICKBOM, 1945; TYMOWSKA and KOBEL, 1972; TYMOWSKA and FISCHBERG, 1973).

Total absolute length of the genome

The total absolute length of the diploid chromosomal complement shows rather little variation between species (fig.1). This might indicate that the length of individual chromosomes varies little among species. In all three examined subspecies of X.laevis, as well as in X.clivii and X.fraseri, the total length of the genome is almost identical. The genome of X.gilli is the shortest, while those of X.borealis and muelleri are longer than the others.

Data from histophotometric studies of nuclear DNA for X.laevis and X.muelleri (OLMO, 1970) reveal a larger difference than the one shown for the total lengths of the two genomes, but the tendency is maintained.

Hypothetical karyotype of Xenopus

The karyotypes of the different Xenopus species are, from the morphological point of view, very similar and closely related. For this reason a schematic common hypothetical idiogram was created for the Xenopus species possessing 36 chromosomes, similar to the one made by Bogart (1972) for the genus Bufo.

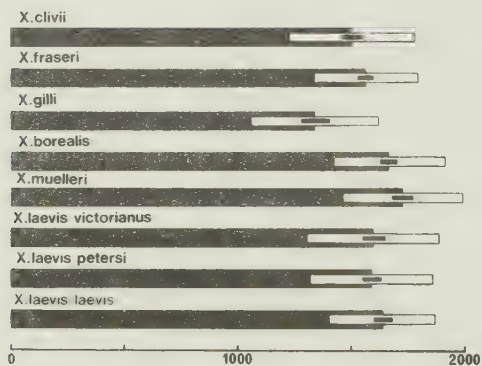


Fig.1 Total length of chromosome complements of *X.l.laevis* (mean = 1640.83), *X.l.petersi* (mean = 1586.15) *X.l.victorianus* (mean = 1601.18), *X.muelleri* (mean = 1729.65) *X.borealis* (mean = 1662.12), *X.gilli* (mean = 1334.60) *X.clivii* (mean = 1565.60), *X.fraseri* (mean = 1507.40) White squares indicate the standard deviation and black squares the standard error of the mean.

The Xenopus idiogram was made on the basis of the mean data of relative lengths and arm ratios from these eight species and subspecies. It also indicates the location of the different secondary constrictions (A-G, fig.2).

The first group of chromosomes is composed of the three longest submetacentric chromosomes (Nos.1 to 3). The second contains four medium submetacentric chromosomes very much alike (Nos. 4 to 7) which carry in the majority of species secondary constrictions (A-F) which allow this group to be split into two or three smaller groups. The third group has only one pair of ~~subac~~rocentric chromosomes (No.8) and the fourth is also made up of a single pair of large metacentric chromosomes (No.9). Two small metacentric chromosomes (Nos.10 and 11) form the fifth group. The last group contains the remaining pairs of chromosomes which are all acrocentric (Nos. 12 to 18). In some species the longest of these chromosomes (No.12) bears secondary constrictions (G), in which case two groups of acrocentric chromosomes arise instead of one.

Secondary constrictions

Due to the similarity of chromosome complements the secondary constrictions play an important role in the cytological classification of Xenopus species. Quite often only the localization of secondary constrictions differentiates certain karyotypes from one another and permits to identify the species cytologically (fig.2).

Symbol of
secondary
constrictions

Present in

A *	-	<u>X.muelleri</u> , <u>X.borealis</u>
B *	-	<u>X.clivii</u>
C **	-	<u>X.gilli</u>
D **	-	<u>X.muelleri</u>
E **	-	<u>X.borealis</u>
F *	-	<u>X.fraseri</u>
G *	-	<u>X.l.laevis</u> , <u>X.l.petersi</u> , <u>X.l.victorinus</u> , <u>X.gilli</u>

* These secondary constrictions are either known or assumed to be nucleolar organizers.

** These three secondary constrictions are assumed to be homologous.

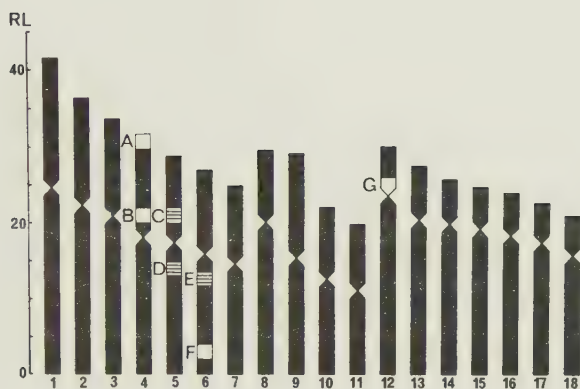


Fig.2 Schematic idiogram of the genus *Xenopus*. A to G represent the localizations of secondary constrictions found in different species of *Xenopus*. The white areas indicate nucleolar organizer constrictions, while lined areas illustrate non specified secondary constrictions.

The karyotypes of the species

Three subspecies of Xenopus laevis have been analyzed: X.laevis laevis, X.l.petersi and X.l.victorianus. The karyotypes of the latter two subspecies are the same as the one described for X.l.laevis (Tymowska and Kobel, 1972). The secondary constrictions of the type G (representing the nucleolar organizer region) appear on the same chromosomes (No.12), are placed on the short arms and connect the centromeres with the satellites (figs. 2,3,6,7). The chromosomes are grouped as shown in the schematic idiogram, one group, composed of chromosome No.12, being added.

Xenopus borealis was once considered a subspecies of X.laevis, but has since become a separate species (TYMOWSKA and FISCHBERG, 1973; FISCHBERG and KOBEL, unpublished). The karyotype of X.borealis is very similar to that of the previously described (TYMOWSKA and KOBEL, 1972) X.muelleri (figs.2,4,6,7). In both species the chromosomes form the same groups, with the exception of group No.5 of muelleri and group No.6 of borealis. The secondary constrictions type E on chromosome No.6 of borealis can be interpreted as being homologous to the constriction D of chromosome No.5 of muelleri. The difference in length of these two secondary constrictions (E and D) affects the lengths of the chromosomes concerned. Chromosome No.5 of muelleri carries the long constriction D while the chromosome of X.borealis, bearing the smaller secondary constriction E, occupies position No.6 instead of No.5.

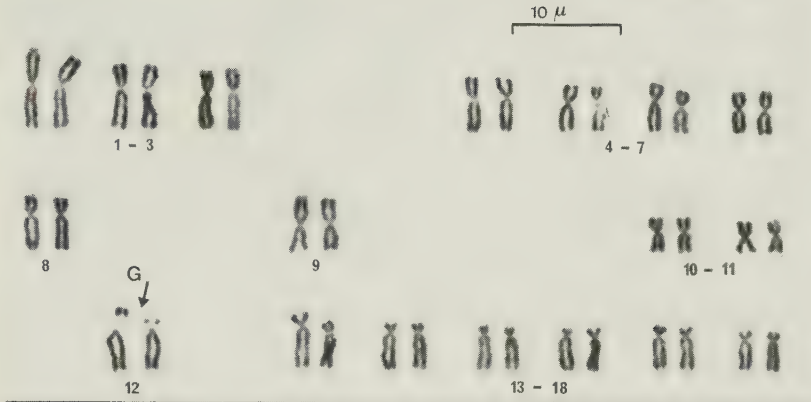
X.laevis laevis*X.laevis petersi**X.laevis victorianus*

Fig.3 Karyotypes of *X.l.laevis*, *X.l.petersi*, *X.l.victorianus* showing morphological groups of chromosomes. The secondary constrictions are indicated by arrows.

In comparison with the schematic idiogram there are in X.borealis two additional groups representing two chromosome pairs (Nos.4 and 6) with secondary constrictions A and E. Pair No.4 bears secondary constrictions A (representing the nucleolar organizer region) which are terminally placed as prolongations of the short arms. The secondary constrictions E of chromosome No.6 appear within the long arms, separated from the centromere by a small condensed region and from the end of the chromosome by long satellites.

The chromosomal analysis of Xenopus gilli has been adversely affected by the small number of available animals. Four secondary constrictions have been found in these karyotypes; two principle ones, on pairs Nos. 5 and 12 (fig.4,6,7) and two rare ones, probably heterozygous on chromosomes Nos. 4 and 5 (fig.4).

The secondary constriction G (representing the nucleolar organizer region) of pair No.12 is similar to that found in the X.laevis group (fig.4) and was observed in 75% of the chromosomes of this pair.

The principal secondary constriction C on pair No.5 is localized on the short arms of the chromosomes, between the small condensed chromatin region, adjacent to the centromere, and the long condensed portion of a satellite. This constriction could be observed in 82.5% of the chromosomes concerned.

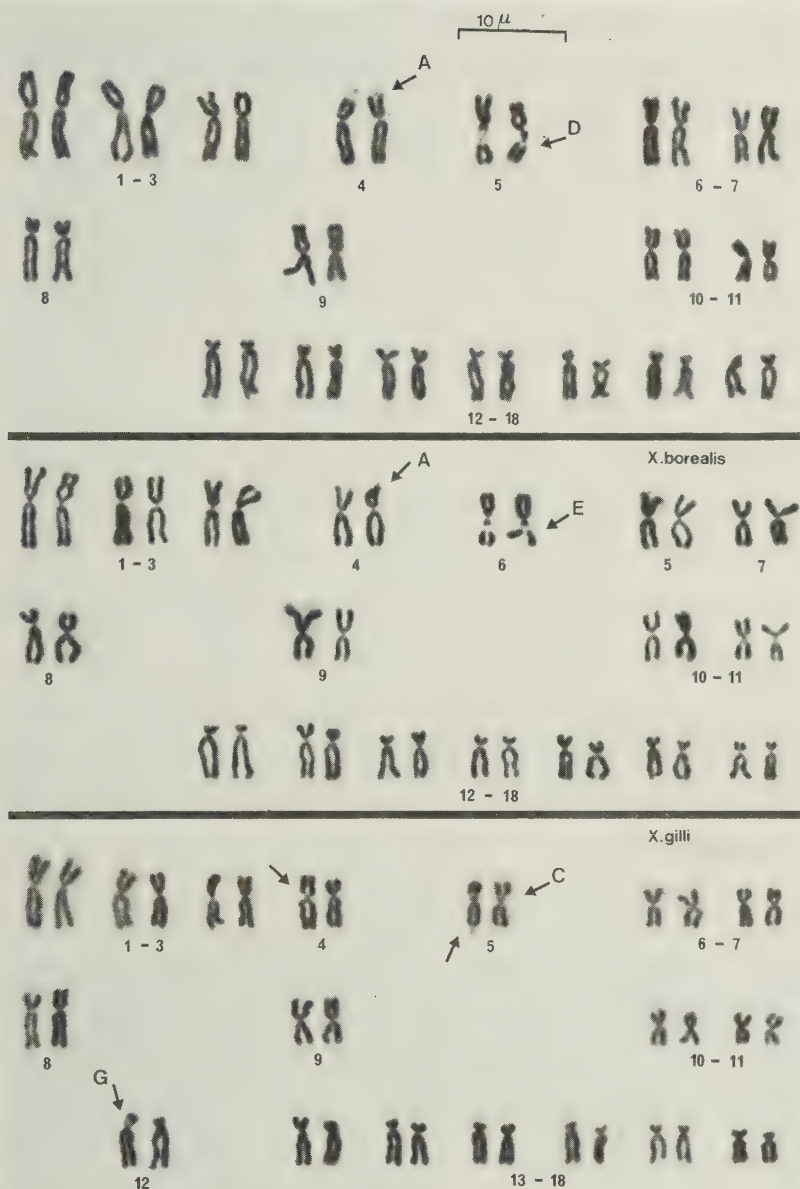


Fig.4 Karyotypes of *X.muelleri*, *X.borealis*, *X.gilli* showing morphological groups of chromosomes. The secondary constrictions are indicated by arrows.

On the karyotype of the analyzed female, an additional secondary constriction was found on one of the chromosomes of pair No.5. This heterozygous constriction is situated at the end of the long arm. The fact that this constriction was found in a female, could lead one to assume the existence of sex-chromosome heteromorphism. However, the small number of animals examined and the presence of another probably heter^ozygous secondary constriction do not permit this conclusion to be drawn.

The secondary constriction of pair No.4 appears on the short arm of the chromosome. attached directly to the centromere and separated from the end of the arm by a long satellite. Although this constriction has been observed on one of the two homologous chromosomes of pair No.4 exclusively, one cannot be certain of its heterozygous character as its occurrence is very rare (in 12.5% of the metaphases only).

The heterozygous constriction on chromosome No.5 and the rare, perhaps also heterozygous, constriction on chromosome No.4 are at the present considered as being cases of either transient physiological expressions of these chromosomes or else as representing anomalies or a polymorphism within a small colony of animals. It has been, therefore, assumed that the general karyotype of X.qilli possesses two pairs of chromosomes (Nos.5 and 12) bearing secondary constrictions (C and G) with each of these pairs forming a group of its own (figs.4,6,7).

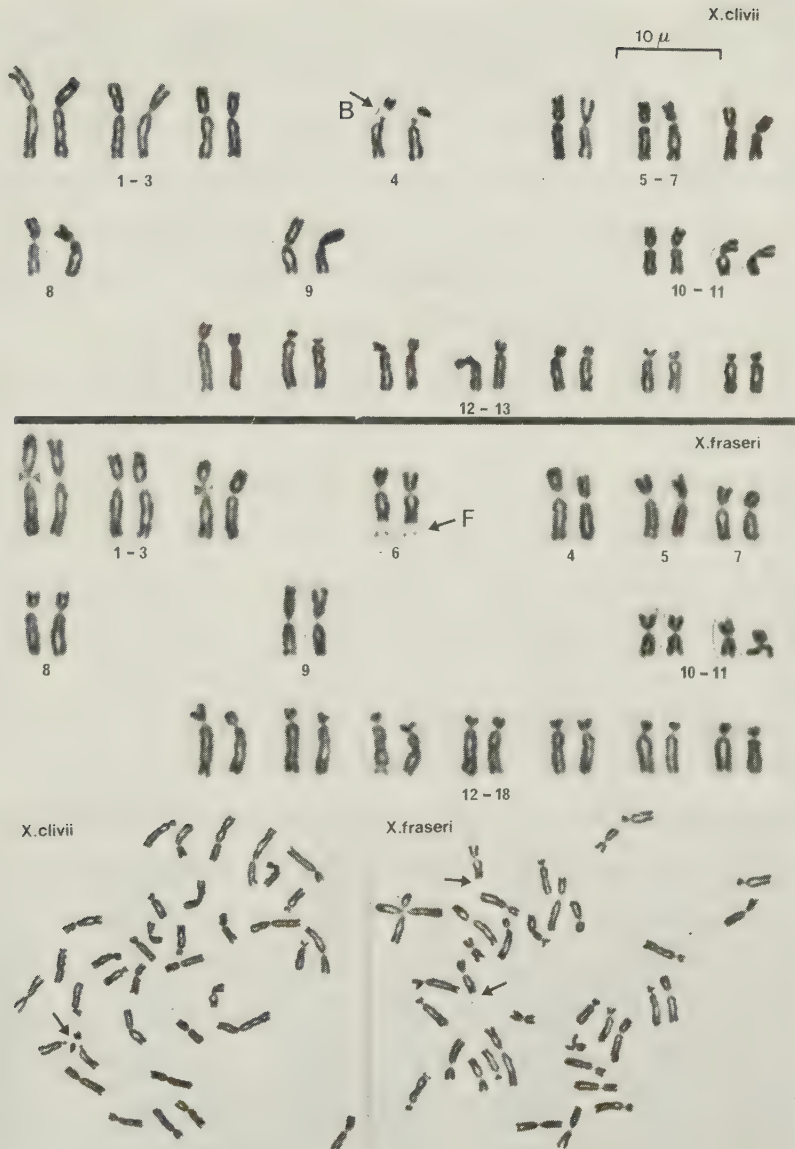


Fig.5 Mitotic chromosomes of *X.clivii* and *X.fraseri* and their karyotypes showing morphological groups of chromosomes. The secondary constrictions are indicated by arrows.

The karyotype of X.clivii possesses one group more than the schematic karyotype. This group is composed of one chromosomal pair (No.4) on which the secondary constrictions B (representing the nucleolar organizer region) are localized on the short arms between long satellites and the small portions of condensed chromatin separating them from the centromeres (fig.5,6,7).

The examined small population of X.fraseri shows two chromosome pairs (No.5 and No.6) which bear secondary constrictions. The one on No.5 is heterozygous. This phenomenon has been found in all four analysed animals of both sexes. The two secondary constrictions of chromosomes Nos. 5 and 6 are quite similar since they appear within the long arms and are separated from the end of the arms by small satellites. The satellites of pair No.6 are slightly smaller than the heterozygous one of chromosome No.5, which permitted their distinction. This heterozygous secondary constriction has been interpreted as a polymorphic translocation in this particular population. Furthermore, it has been concluded that the general karyotype of X.fraseri is characterized by a secondary constriction which appears on chromosome pair No.6. This pair forms a group by itself and is typical of this species only (figs.5,6,7).

The chromosome analysis of these eight species and subspecies of Xenopus has not shown any morphological differences which indicate the existence of sex-chromosome dimorphism. The particular case of a heterozygous female

in X.gilli, however, should not be omitted and further studies are obviously

Table I. Morphometric characteristics of the chromosomes of X.l.petersi and X.l.victorinus

Pair No.	Relative length (o/oo of 2n set)			Arm ratio ($\frac{\text{short}}{\text{long}} \times 100$)		
	Mean	SD	SE	Mean	SD	SE
<u>X.l.petersi</u> (2 ♂♂, 2 ♀♀; 40 mitoses)						
1	41.82	2.67	0.29	66.50	7.18	0.80
2	36.03	1.73	0.19	60.21	5.41	0.60
3	33.69	1.68	0.18	58.15	6.30	0.70
4	30.49	1.94	0.21	60.39	7.22	0.80
5	26.55	1.50	0.16	67.67	8.62	0.96
6	25.40	1.07	0.11	67.65	7.99	0.89
7	24.29	1.24	0.13	68.97	9.05	1.01
8	30.29	2.28	0.25	47.38	5.11	0.57
9	29.05	1.75	0.19	88.03	8.02	0.89
10	21.32	1.93	0.21	86.47	7.83	0.87
11	19.47	1.37	0.15	86.19	11.12	1.24
12	32.22	2.78	0.31	30.79	7.80	0.87
13	28.88	2.03	0.22	35.14	5.41	0.60
14	26.43	1.21	0.13	26.88	5.46	0.61
15	25.37	1.26	0.14	24.83	4.56	0.50
16	24.25	1.10	0.12	27.58	4.99	0.55
17	23.15	1.03	0.11	30.45	5.87	0.65
18	21.42	1.35	0.15	32.86	6.14	0.68
<u>X.l.victorinus</u> (2 ♂♂, 2 ♀♀; 37 mitoses)						
1	42.27	3.34	0.38	71.30	6.65	0.77
2	36.98	2.81	0.32	62.89	6.31	0.73
3	34.43	1.85	0.21	62.12	6.26	0.72
4	30.83	2.22	0.25	63.82	8.19	0.95
5	27.02	2.51	0.29	65.80	8.50	0.98
6	25.75	1.86	0.21	68.13	9.33	1.08
7	23.63	3.14	0.36	70.31	10.76	1.25
8	30.17	2.90	0.33	51.67	9.25	1.07
9	29.91	2.33	0.27	91.49	6.28	0.73
10	20.80	1.65	0.19	68.47	9.29	1.08
11	19.59	2.67	0.31	59.09	8.23	0.95
12	32.63	3.73	0.43	34.62	9.71	1.12
13	28.08	1.78	0.20	34.07	4.71	0.54
14	26.26	2.05	0.23	26.09	4.53	0.52
15	24.73	1.22	0.14	26.10	5.52	0.64
16	23.97	1.22	0.14	27.06	7.46	0.86
17	22.99	1.55	0.18	27.42	6.03	0.70
18	20.65	1.57	0.18	33.37	8.27	0.96

Table II.

Morphometric characteristics of the chromosomes
of X.borealis and X.gilli

Pair No.	Relative length (o/oo of 2n set)			Arm ratio ($\frac{\text{short}}{\text{long}} \times 100$)		
	Mean	SD	SE	Mean	SD	SE
<u>X.borealis</u> (2 ♂♂, 2 ♀♀; 40 mitoses)						
1	41.17	2.33	0.26	71.96	5.38	0.60
2	36.04	1.76	0.19	68.33	8.30	0.92
3	33.65	1.58	0.17	65.73	6.49	0.72
4	33.77	2.52	0.28	74.63	10.44	1.16
5	29.41	1.80	0.20	68.79	8.35	0.93
6	27.95	1.71	0.19	90.60	7.09	0.79
7	26.33	1.83	0.20	78.78	11.04	1.23
8	28.91	2.30	0.25	51.24	7.65	0.85
9	29.36	1.93	0.21	90.63	6.82	0.76
10	24.36	1.35	0.15	85.11	7.85	0.88
11	21.51	1.58	0.17	87.51	9.44	1.05
12	27.64	1.71	0.19	22.18	3.86	0.43
13	27.29	2.49	0.27	41.64	6.85	0.76
14	24.25	1.46	0.16	39.52	7.64	0.85
15	23.82	1.12	0.12	33.33	5.98	0.66
16	23.02	1.21	0.13	33.87	7.36	0.82
17	21.64	1.33	0.14	38.85	8.12	0.90
18	19.89	1.08	0.12	37.91	6.34	0.71
<u>X.gilli</u> (1 ♂, 1 ♀; 20 mitoses)						
1	41.78	2.91	0.46	67.87	8.27	1.30
2	37.00	2.43	0.38	63.72	7.58	1.19
3	34.42	1.61	0.25	60.34	7.74	1.22
4	31.24	1.65	0.26	69.29	8.80	1.39
5	30.85	3.75	0.59	76.31	10.06	1.59
6	25.53	1.56	0.24	75.28	8.09	1.28
7	23.65	1.30	0.20	73.44	8.66	1.37
8	30.70	2.07	0.32	51.23	5.90	0.93
9	28.69	2.54	0.40	90.06	6.60	1.04
10	21.33	2.01	0.31	85.35	9.47	1.49
11	19.26	1.98	0.31	80.68	12.36	1.95
12	31.10	3.29	0.52	30.14	9.47	1.49
13	28.04	1.95	0.30	34.40	4.73	0.74
14	25.45	1.44	0.22	29.30	8.62	1.36
15	24.78	1.12	0.17	26.95	7.00	1.10
16	23.53	1.18	0.18	29.50	5.40	0.85
17	22.41	1.12	0.17	33.40	5.80	0.91
18	20.30	1.81	0.28	34.55	7.07	1.11

Table III. Morphometric characteristics of the chromosomes of X.clivii and X.fraseri

Pair No.	Relative length (o/oo of 2n set)			Arm ratio ($\frac{\text{short}}{\text{long}} \times 100$)		
	Mean	SD	SE	Mean	SD	SE
<u>X.clivii</u> (2 ♂♂, 2 ♀♀; 40 mitoses)						
1	41.69	2.48	0.27	67.99	5.97	0.66
2	36.67	2.04	0.22	63.82	7.16	0.80
3	33.65	1.77	0.19	62.03	6.82	0.76
4	31.32	2.30	0.25	72.70	11.41	1.27
5	30.28	1.55	0.17	65.43	7.09	0.79
6	26.92	1.30	0.14	70.68	6.18	0.69
7	25.34	0.87	0.09	71.97	7.33	0.82
8	30.70	2.22	0.24	50.83	4.98	0.55
9	28.64	2.04	0.22	89.19	8.68	0.97
10	23.90	1.08	0.12	74.18	6.51	0.72
11	20.34	1.32	0.14	79.64	9.04	1.01
12	27.84	1.54	0.17	18.90	3.78	0.42
13	27.75	1.48	0.16	36.59	4.88	0.54
14	25.75	1.11	0.12	30.01	4.18	0.46
15	24.45	1.00	0.11	31.47	5.17	0.57
16	23.47	1.14	0.12	34.49	6.39	0.71
17	21.70	1.21	0.13	29.49	5.44	0.60
18	20.19	1.24	0.13	30.67	5.00	0.55
<u>X.fraseri</u> (2 ♂♂, 2 ♀♀; 40 mitoses)						
1	42.91	3.43	0.38	65.37	6.60	0.73
2	36.48	2.22	0.24	61.03	6.96	0.77
3	33.98	1.90	0.21	61.09	6.47	0.72
4	30.82	1.84	0.20	61.41	6.68	0.74
5	28.70	2.15	0.24	56.22	7.50	0.83
6	27.31	2.17	0.24	55.29	8.28	0.92
7	24.91	1.67	0.18	65.52	6.57	0.72
8	29.56	1.80	0.20	45.55	5.86	0.65
9	29.35	1.85	0.20	89.91	7.54	0.84
10	19.85	1.64	0.18	78.99	10.22	1.14
11	17.50	1.56	0.17	78.44	10.39	1.16
12	29.44	1.96	0.21	30.26	7.67	0.85
13	27.69	1.82	0.20	29.10	7.08	0.79
14	26.35	1.35	0.15	26.10	5.17	0.57
15	25.31	1.23	0.13	26.34	5.39	0.60
16	24.56	1.34	0.15	26.05	5.86	0.65
17	23.39	1.49	0.16	27.97	5.30	0.59
18	21.29	1.23	0.13	33.03	7.07	0.79

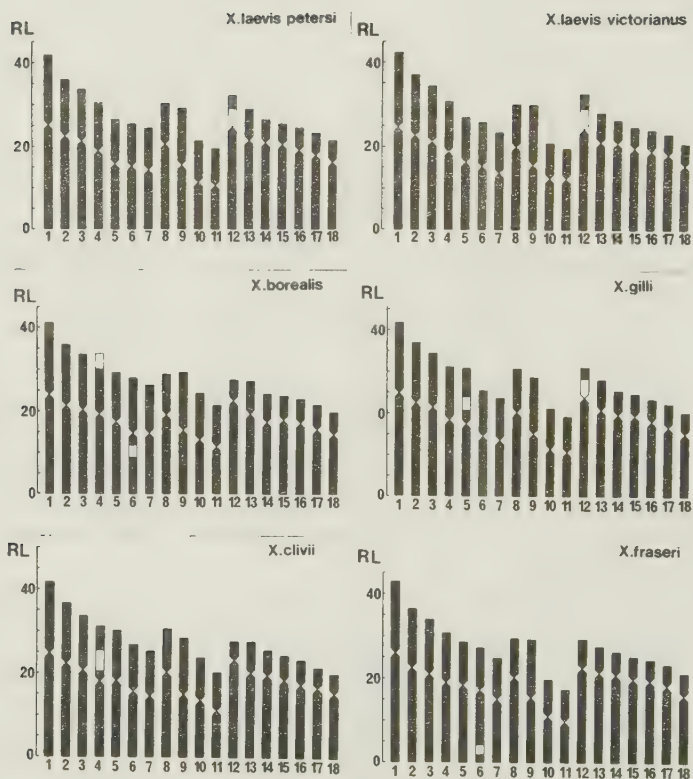


Fig.6 Idiograms of *X.l.petersi*, *X.l.victorianus*, *X.borealis*, *X.gilli*, *X.clivii*, *X.fraseri* based on the measurements of relative length and arm ratios.

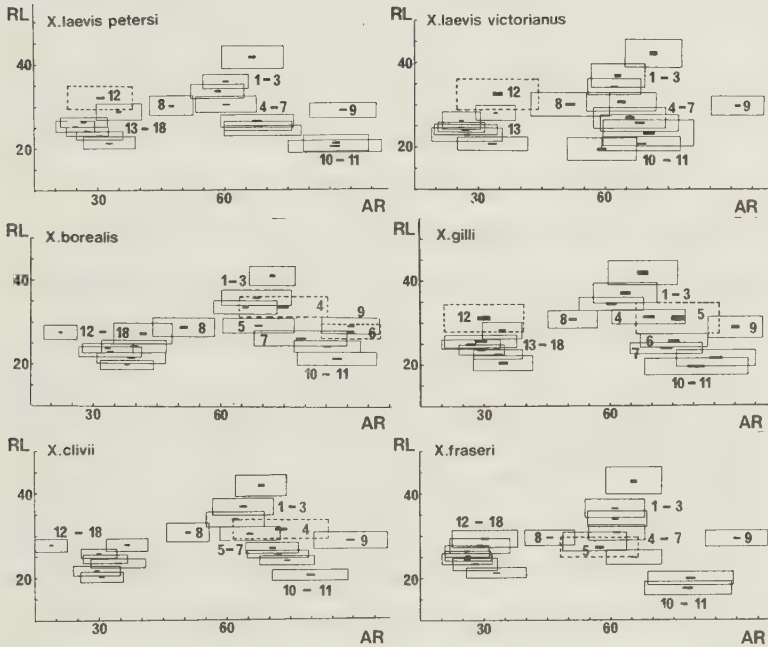


Fig.7 Two-dimensional karyograms of *X.l.petersi*, *X.l.victorianus*, *X.borealis*, *X.gilli*, *X.clivii*, *X.fraseri* showing morphological groups of chromosomes based on measurements of RL and AR. Black squares indicate the error of the mean and white squares the standard deviation. Chromosomes with secondary constrictions are designated by dotted lines.

DISCUSSION

Nucleolar organizer and chromosomal association.

The cytological analysis (KAHN, 1962) of the Xenopus laevis Oxford nucleolar mutant (ELSDALE et al., 1958; FISCHBERG and WALLACE, 1960) showed that the secondary constriction (G) of the satellite chromosome No.12 plays the role of the nucleolar organizer. Furthermore, these studies have been confirmed by Pardue et al. (1973) by "in situ" hybridization of rRNA to the complementary genes.

The mitotic plates of X.laevis reveal that the two homologous chromosomes (No.12) possessing nucleolar organizers show chromosomal association (TYMOWSKA and KOBEL, 1972). It is supposed that this association is a result of the prior attachment of nucleolar constrictions to fused nucleoli (WALLACE, 1963). The assumption that the chromosome pairs showing association in X.muelleri are those which possess nucleolar organizers is confirmed by "in situ" hybridization (PARDUE, 1974). Human karyotypes demonstrate several pairs of acrocentric chromosomes containing sites of nucleolar organizers with a high frequency of satellite association (COHEN and SHAW, 1967; COOKE, 1971 and 1972; HENDERSON et al., 1972; SCHMID and KRONE, 1974). The phenomenon of homologous association can, therefore, be considered as an indication that these secondary constrictions contain nucleolar organizers.

Similar behaviour of chromosomal association was observed in all examined species (Table IV) and from these data it can be established for each of the eight species which of the chromosomes bear nucleolar constrictions (fig.8).

The nucleolar organizer in the subspecies X.l.victorianus and X.l.petersi is placed as in X.laevis on chromosome No.12. The chromosome association can be observed in 52%, 22% and 51% of the analysed mitoses, respectively (fig.8a,b,c).

X.borealis possesses a similar pattern of secondary constrictions as X.muelleri in which the constrictions on chromosome pair No.4 represent the nucleolar organizers (TYMOWSKA and KOBEL, 1972; PARDUE, 1974). Moreover, in both cases the constriction is placed terminally and is associated in 50% of the metaphases in X.muelleri and in 43% of those in X.borealis (fig.8e,f). It is therefore assumed that this constriction on chromosome No.4 of X.borealis is the nucleolar organizer.

In X.gilli only one pair (No.12) shows a homologous association in 40% of mitoses (fig.8d). This secondary constriction (G) is morphologically similar to the nucleolar constriction of the X.laevis group and probably plays the same role.

X.clivii has, as judged by the chromosomal association (50%), a nucleolar organizer within the short arm of chromosome pair No.4 (fig.8,g).

Table IV. Frequency of association of homologous chromosomes bearing secondary constrictions in metaphase plates.

	Number of metaphases with: homologs associated homologs separated		Number of metaphases
<u>X.l.petersi</u>			
pair No. 12 (G)	21	19	40
<u>X.l.victorinus</u>			
pair No. 12 (G)	8	29	37
<u>X.borealis</u>			
pair No. 4 (A)	17	23	40
pair No. 6 (E)	--	40	40
<u>X.gilli</u>			
pair No. 5 (C)	--	20	20
pair No.12 (G)	8	12	20
<u>X.fraseri</u>			
pair No.6 (F)	9	31	40
<u>X.clivii</u>			
pair No. 4 (B)	20	20	40



Fig.8 Chromosomes of eight *Xenopus* species and subspecies showing associations.
 a) to d) chromosomes of pairs No.12 of *X.l.laevis*, *X.l.petersi*, *X.l.victorianus*, *X.gilli* respectively.
 e) f) chromosomes of pairs No.4 of *X.muelleri* and *X.borealis*.
 g) chromosomes of pair No.4 of *X.clivii*. h) chromosomes of pair No.6 of *X.fraseri*.
 G,A,B,F symbols of secondary constrictions.

X.fraseri possesses its own type of secondary constriction (F) which demonstrates association (fig.8h) not only between the two homologs of pair No.6 but also with the heterozygous constriction of pair No.5. This indicates that the peculiarity of the analysed fraseri population is due to a nucleolar mutation (translocation) and that these three secondary constrictions are in reality nucleolar organizers. As to be expected, three nucleoli have been observed in X.fraseri nuclei (FISCHBERG, personal communication).

The percentage of chromosomal associations for most of the species investigated lies between 40-50%. It is not known to what the lower percentage of association in X.l.victorinus (22%) and in X.fraseri (23%) is due.

Length of secondary constrictions

Since a correlation generally exists between chromosome size and DNA value, (MIRSKY and ROS, 1951; ULLERICH, 1966; OHNO and ATKIN, 1966; ROMANINI, 1973), it was of interest to measure the length of secondary constrictions in the different species. The results are presented in Table V and fig.9. The length of nucleolar organizers varies rather little among the species studied with the exception of X.fraseri. This species shows a nucleolar constriction length which is only half of that of the other species.

In X.laevis the nucleolar organizer contains 450-650 copies of genes coding for rRNA, (WALLACE and BIRNSTIEL, 1966) Another species possesses the same number of copies coding for 18 and 28 S RNA and these sequences are not detectably different from those of laevis except for the DNA spacer, (BROWN et al. 1972; BROWN and SUGIMOTO, 1974). Presumably, provided one can base the number of cistrons on the length of a secondary constriction, each species will possess a number of rRNA cistrons corresponding to the length of its nucleolar organizers.

Cytotaxonomic problems

The karyotype of the Xenopus species analysed in this paper is remarkably uniform in number, size and shape. The only major morphological chromosomal variation observed concerns morphology and position of secondary

Table V. Absolute length of secondary constrictions

Name of species	Number of chromosome pair	Symbol of secondary constriction	n	Mean	SD	SE
<i>X.l.laevis</i>	12	G	72	7.27	2.62	0.30
<i>X.l.petersi</i>	12	G	61	7.60	2.17	0.27
<i>X.l.victorinus</i>	12	G	54	8.66	3.34	0.45
<i>X.muelleri</i>	4	A	57	7.57	2.60	0.34
	5	D	76	12.81	4.59	0.52
<i>X.borealis</i>	4	A	55	5.94	2.29	0.30
	6	E	80	4.78	1.60	0.17
<i>X.gilli</i>	5	C	33	4.21	1.42	0.24
	12	G	30	6.96	3.38	0.61
<i>X.clivii</i>	4	B	80	7.67	2.74	0.30
<i>X.fraseri</i>	6	F	54	3.12	1.72	0.23

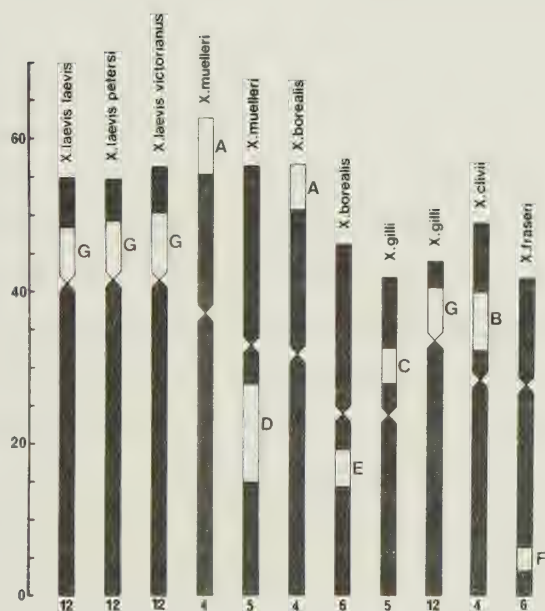


Fig.9 Idiogram of the chromosomes carrying secondary constrictions of each analysed species, based on the absolute measurements (Table V).

constrictions. Other possible evolutionary changes which are single or multiple gene mutations at a particular loci, such as slight deletions, duplications, translocations, paracentric or pericentric inversions involving small amounts of chromatin in the Xenopus complement might have occurred, but these cannot be detected by measurements of relative length and arm ratios. The existence of small differences in the total length of the genome of the different Xenopus species is probably due to a variable amount of heterochromatization; it has, however, not been possible to identify all heterochromatic regions. In many Anuran genera a centric fusion and fission of chromosomes is regarded as the main mechanisms of phylogenetic differentiation (MORESCALCHI, 1968b, 1973; BOGART, 1970, 1973; SCHEEL, 1971). In the genus Xenopus, the effect of such mechanisms could not be detected with certainty as having played a role during the evolutionary changes. The analysis of chromosomal rearrangements has, therefore, to consider the number, morphology, localization and the role of secondary constrictions. A similar situation occurs in the genus Bufo in which uniform karyotypes can usually be distinguished by secondary constrictions (BOGART, 1972).

Some of the Xenopus secondary constrictions (A,B,F,G) are known to be, or interpreted as nucleolar organizers. It should be stressed that these regions appear to manifest a high mutability and possess, therefore,

a strong evolutionary potentiality. This is demonstrated by the following mutations: a) the well-known Oxford nuclear marker (O nu) for X.laevis, b) the nucleolar mutation in the polymorphic colony of X.fraseri described in the present study and c) the recently reported case of a nucleolar organizer mutation in X.borealis, which is presumably due to a reciprocal translocation, revealing an additional, heterozygous nucleolar constriction (JOTTERAND and FISCHBERG, 1974). In addition, on examining the idiogram of chromosomes bearing secondary constrictions (fig.9) it would be easy to postulate several possible rearrangements which could have taken place during evolution. For instance, a paracentric inversion on the short arm of chromosome No.4 of X.borealis or muelleri could result in a new localization of the secondary constriction within an arm, such as in X.clivii, with a simultaneous loss of chromatin.

It cannot be determined whether a particular constriction (except that of the nucleolar organizer regions) is definitely homologous between two species, however, it is evident that highly compatible species have similar secondary constrictions. It could, therefore, be concluded from the results of karyotype analysis that : a) subspecies such as X.l.laevis, X.l.petersi, X.l.victorianus show a close interrelationship; b) that this group stays in systematic proximity of X.gilli which possesses a similar nucleolar organizer; c) that the other constriction of X.gilli relates to the group of X.muelleri and borealis which demonstrate a similar pattern of constrictions.

The shorter length of the gilli constriction influences its arm ratio and is for this reason found within the short arm instead of the long arm such as in muelleri and borealis. Because of this the karyotype of X.gilli could be taken as an intermediate between the group of X.laevis and X.muelleri. d) if the hypothesis of a paracentric inversion involving chromosome No.4 which reveals homology between the nucleolar constrictions A and B, can be accepted, then X.clivii could in some respects be regarded as a species close to the group of X.muelleri. e) the particular nucleolar constriction which is only found in X.fraseri indicates that there is little direct relationship with the other species.

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Running head

TYMOWSKA, FISCHBERG, TINSLEY, The Tetraploid species
Xenopus vestitus

Title

The karyotype of the tetraploid species
Xenopus vestitus Laurent (Anura : Pipidae).¹⁾

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ABSTRACT

Xenopus vestitus possesses 72 chromosomes, whereas in the majority of known Xenopus species there are $2n = 36$. During meiosis, 36 bivalents are observed at metaphase I and at metaphase II there are 36 chromosomes. Arranged according to size and centromere position, the chromosomes form the same basic morphological groups which are typical of the genus Xenopus. However, the groups are composed of quartets of four similar chromosomes instead of the usual diploid pairs of homologues. The exception to this arrangement involves chromosomes bearing secondary constrictions which are in X. vestitus represented by two different pairs of homologues one of which shows, in 39% of the observed mitoses, somatic association and is, therefore, considered to carry the nucleolar organizer. X. vestitus represents either a case of ancient autotetraploidy, or more likely one of allotetraploidy of more modern origin.

INTRODUCTION

In the last few years several examples of anuran polyploidy have been reported. In the subfamily Ceratophryinae there are two known octoploid species of the genus Ceratophrys, C.dorsata (see BEÇAK et al., 1967) and C.ornata (see BOGART, 1967), one tetraploid species of the genus Odontophrynus, O.americanus (see BEÇAK et al., 1966, 1967; BOGART, 1967). In the subfamily Leptodactylinae there are two tetraploid species of the genus Pleurodema, P.bibronii and P.kriegi (see BARRIO and RINALDI DE CHERI, 1970). In the family Hylidae there exist one tetraploid species of the genus Hyla, H.versicolor (see WASSERMANN, 1970), and another tetraploid species of the genus Phyllomedusa, P.burmeisteri (see BEÇAK et al., 1970). Recently one hexaploid species has been found in the family Pipidae, Xenopus ruwenzoriensis (see TYMOWSKA and FISCHBERG, 1973). This occurrence of polyploid species among Anura indicates that polyploidization plays a certain role in the evolution of this group of animals. It is likely that more polyploid species will be discovered among anurans.

Laurent (1972), working with museum collections from the former Albert National Park (Parc National des Virunga), Zaïre, reported a new species of the African clawed toad Xenopus, X.vestitus. The description of a new species X.kigesiensis by Tinsley (1973), based on living

specimens from Lake Mutanda, S.W. Uganda, was published shortly after Laurent's account. Comparison of the respective type collections has shown that X.kigesiensis and X.vestitus are synonymous (TINSLEY, 1975).

The distribution of X.vestitus overlaps with that of three other Xenopus species (TINSLEY, 1975) but morphologically X.vestitus is characterized by its ventral patterns and small body dimensions (LAURENT, 1972) as well as by its relatively pointed snout, small eyes and short limbs and digits (TINSLEY, 1973,1975).

Recent work by Fischberg, Kobel and Tymowska (see references) has shown that cytological studies provide a valuable tool in the assessment of evolutionary relationships between the Xenopus species; the aim of the present paper is to describe the karyotype of X.vestitus.

MATERIAL AND METHODS

Altogether seven X. vestitus have been used for this cytological study. Two of which (1♀ and 1♂) were collected by R.C.T. in 1968 at Lake Mutanda in South-west Kigezi, Uganda. Their chromosomes were measured and analysed in great detail. One female and one male offspring of these two animals were also checked for their chromosome number and chromosome morphology. In addition 1♂ from Lake Muleke, just east of Lake Mutanda, 1♀ from Echuya Forest, south-east of L. Muleke and 1♀ from Shama in East Rwanda near the Tanzanian border were also checked for chromosome number and morphology. The last three animals were collected by M.F., R.C.T., Dr. H.R. Kobel and Dr. L. Du Pasquier in 1975.

The direct chromosome preparations were made by the method previously described (TYMOWSKA and KOBEL, 1972). Ten mitotic plates were examined for each of the animals and chromosomes were arranged according to their size and centromere position, applying the classification of Levan et al. (1964) and the commonly used nomenclature. The measurements of chromosomes are expressed as absolute length, relative length (RL) and arm ratio (AR) (Table I, idiograms figs.3, 5 and two-dimensional karyograms fig.4).

RESULTS

Number of chromosomes

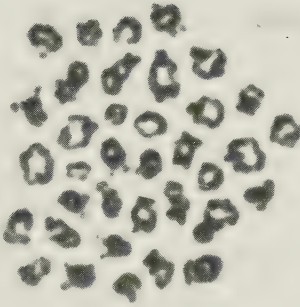
In contrast to the majority of the Xenopus species which possess 36 chromosomes, X. vestitus has 72 chromosomes. During male meiosis 36 bivalents are observed at metaphase I and in metaphase II there are 36 chromosomes (fig.1,2).

Karyotypes

The 72 chromosomes of X. vestitus form the same basic morphological groups as illustrated by the hypothetical idiogram for all those Xenopus species which possess 36 chromosomes (TYMOWSKA, submitted to press), the only difference being that each numbered chromosome is represented by a set (quartet) of four similar chromosomes instead of the usual two homologues. However, there are two quartets of 4 chromosomes which are visibly not homogeneous (Nos.2 and 12): in each of these only two of the 4 similar chromosomes are bearing secondary constrictions.

The first group of chromosomes of the whole complement is composed of the three longest submetacentric quartets (fig.2, nos.1 to 3). Two chromosomes only of No.2 bear secondary constrictions which are placed within the long arms, between two long segments of condensed chromatin. The second group is formed by four medium-sized submetacentric quartets (Nos.4 to 7). Only one quartet of chromosomes

a



b



Fig.1a) Metaphase of first spermatocyte division of X.vestitus showing 36 bivalents.

b) Metaphase of second spermatocyte division showing 36 chromosomes.

(No.8) belongs to the third group which is subacrocentric. One quartet of large metacentric chromosomes (No.9) forms the next group and two small metacentric chromosome quartets (Nos.10 and 11) make up the fifth group. The 12th quartet, composed of the longest acrocentric chromosomes, is heterogeneous, only two homologous chromosomes of the 4 similar ones carrying secondary constrictions; these appear terminally on the short arms. Within the last group fall all acrocentric chromosomes from Nos.12 to 18 (fig.2,3,4).

There are no morphological differences between the X.vestitus chromosomes which could be interpreted as chromosomal sex-dimorphism.

Secondary constrictions in respect to nucleolar organizers.

In X.vestitus two homologues of the quartet No.12 possess terminally located secondary constrictions which show somatic associations in 39% of the cells examined. A similar association, in some cases even more frequent, has been observed in all Xenopus species (TYMOWSKA and KOBEL, 1972; TYMOWSKA, 1973; TYMOWSKA, submitted to press). In X.laevis and X.muelleri the secondary constrictions participating in association are nucleolar organizers (PARDUE et al., 1973; PARDUE, 1974). By analogy with this particular behaviour all secondary constrictions occurring in the other Xenopus species which demonstrate somatic association are considered as nucleolar regions and this interpretation may be applied in the case of the two terminal constrictions bearing chromosomes No. 12 in X.vestitus.

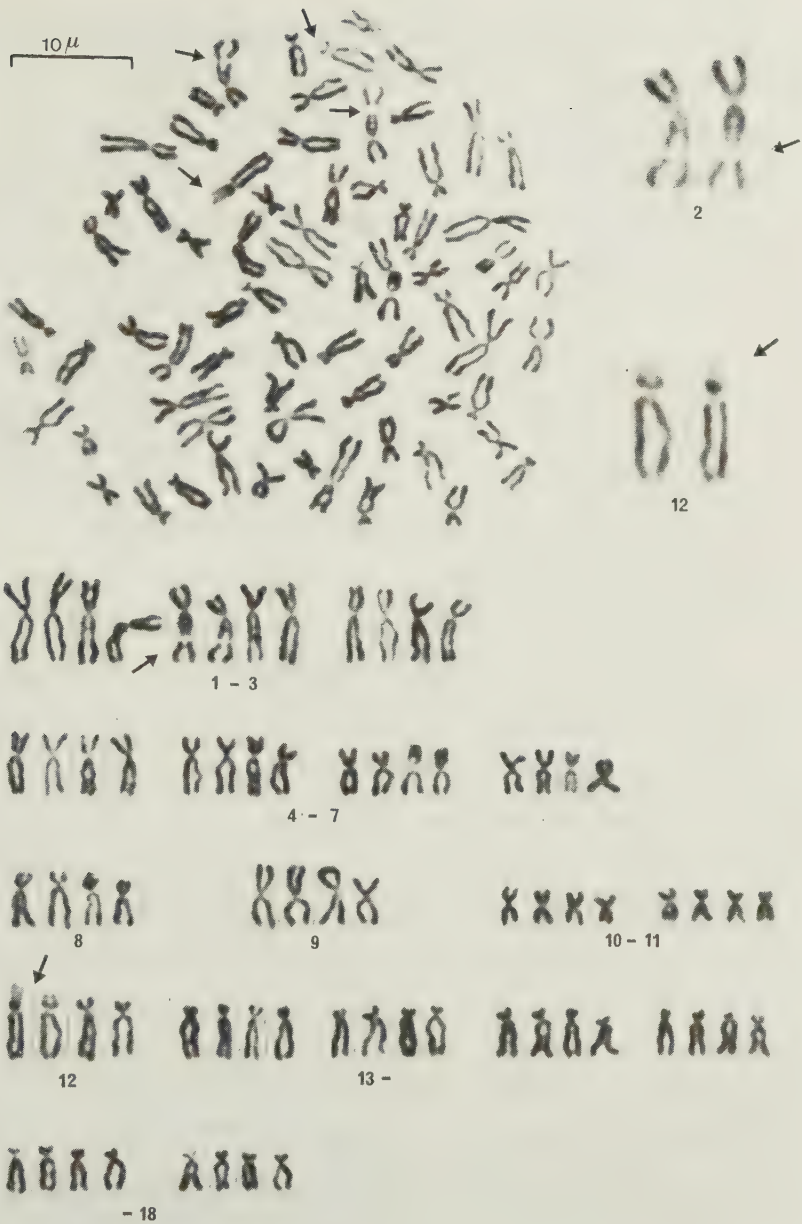


Fig. 2) Mitotic chromosomes and karyotype of *X. vestitus*. Secondary constrictions are indicated by arrows.

The other secondary constrictions of X.vestitus which are located on two chromosomes of quartet No.2 were never seen to be associated, and their frequency of appearance is smaller (50%) than that of the nucleolar constrictions on chromosome No.12 (visible in 82,5%).

DISCUSSION

Knowingly neglecting the diploid chromosome number of X.tropicalis ($2n = 20$) (TYMOWSKA, 1973) which suggests an original basic chromosome number of 9 or 10 and an ancient tetraploidy of most species with $2n = 36$ chromosomes, we accept 18 as the basic number for the genus Xenopus (TYMOWSKA and FISCHBERG, 1973). Therefore X.vestitus possessing 72 chromosomes is interpreted as a tetraploid species.

The chromosomes of this species fit into quartets of four morphologically similar chromosomes. These quartets can be classified into these groups which are typical of Xenopus (TYMOWSKA, submitted to press) and which is also an indication of tetraploidy (figs.2,3,4). However, in the first meiotic metaphase 36 bivalents and no multivalents are observed (fig.1), the chromosomes behaving as in a diploid organism. Moreover, the chromosomes which bear secondary constrictions are represented by two homologues only and reveal for this reason a visible heterogeneity in their own quartets (figs.2,3,4).

Similar situations have been reported for two tetraploid species of Pleurodema. In P.kriegi and P.bibronii (by BARRIO and RINALDI DE CHIERI, 1970) meiotic metaphase I shows only bivalents and mitotic karyotypes display only two homologous chromosomes with secondary constrictions

Table I. Morphometric characteristics of X. vestitus chromosomes (1♂, 1♀; 20 mitoses).

Quartet No.	Relative length (o/oo of 4n set)			Arm ratio ($\frac{\text{short}}{\text{long}} \times 100$)		
	Mean	SD	SE	Mean	SD	SE
1	21.86	1.89	0.21	70.16	8.03	0.89
2	18.86	1.23	0.13	58.50	7.53	0.08
3	17.52	1.06	0.11	55.52	7.76	0.86
4	16.02	1.06	0.11	60.07	8.37	0.93
5	14.17	1.17	0.13	59.58	9.04	1.01
6	12.23	0.66	0.07	69.20	9.63	1.07
7	10.92	1.62	0.18	71.15	12.37	1.38
8	15.14	1.30	0.14	41.42	6.57	0.37
9	15.32	1.10	0.12	89.65	8.93	0.99
10	9.42	0.75	0.08	84.25	12.05	1.34
11	8.33	0.78	0.08	74.05	12.50	1.39
12	16.33	2.02	0.22	32.34	11.90	1.33
13	14.16	0.76	0.08	28.79	5.08	0.56
14	13.08	0.57	0.06	26.32	4.38	0.48
15	12.42	0.50	0.05	28.88	4.85	0.54
16	11.92	0.57	0.06	29.72	5.44	0.60
17	11.20	0.51	0.05	30.35	5.69	0.63
18	10.18	0.84	0.08	35.40	7.70	0.86

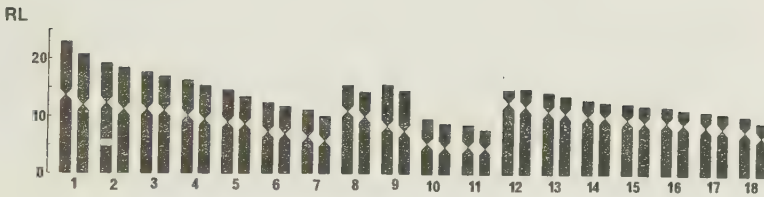


Fig. 3) Idiogram of *X. vestitus* based on the measurements of relative length and arm ratio. The mean length of the two larger and the two smaller chromosomes of each quartet was calculated separately.

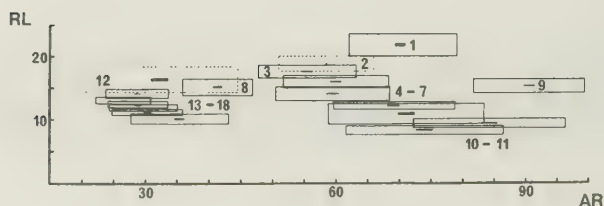


Fig. 4) Two-dimensional karyogram of X.vestitus showing the morphological groups of chromosomes based on measurements of RL and AR and calculated for each of the quartets. Black rectangles indicate the standard error of the mean and white rectangles the standard deviation. Chromosomes carrying secondary constrictions are designated by dotted lines.

instead of four. Such polyploid situations may be interpreted as cases of allotetraploidy, derived by duplication of a hybrid genome.

The strong similarity of the karyotypes of the different species of the genus Xenopus may be the reason why the chromosomes of this polyploid species, although probably being an allotetraploid one, still show general homogeneity within the quartets of which the chromosome groups are composed.

Some cases of tetraploidy in other species, for example Odontophrynus americanus (see BEČAK, 1966), Phyllomedusa burmeisteri (see BEČAK et al., 1970) and Hyla versicolor (see WASSERMANN, 1970), show different aspects from that in Xenopus. In meiosis, multivalents are regularly observed. With the exception of H.versicolor sets of four homologues carrying secondary constrictions are seen at mitosis. These species are considered to be autotetraploids which have arisen by the duplication of the ancestral genome.

In comparison with X.laevis, the total absolute length of the chromosome complement of X.vestitus is not double, as might be expected, but only 67% greater than that of the former species (fig.5). This is probably due to a stronger spiralisation or greater condensation of the X.vestitus chromosomes, as Thiébaud and Fischberg (in press) showed that the DNA amount of X.vestitus erythrocyte nuclei

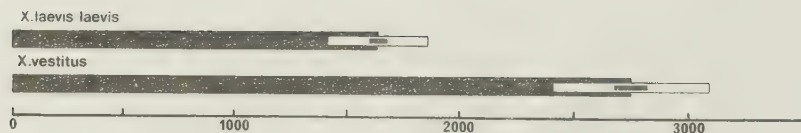


Fig. 5) Total length of chromosome complements of *X.laevis laevis* (mean = 1601.83) and *X.vestitus* (mean = 2756.25). White rectangles indicate the standard deviation and black rectangles the standard error of the mean.

was exactly twice that of comparable nuclei of X.laevis.

The examination of the karyotype of both sexes has failed to reveal any sex-chromosomal dimorphism not only for X.vestitus but also for all other known Xenopus species (MIKAMO and WITSCHI, 1966; TYMOWSKA and KOBEL, 1972; TYMOWSKA, 1973; TYMOWSKA and FISCHBERG, 1973). It may be assumed that in these lower vertebrates, sex chromosomes are still in such an undifferentiated stage that they allow polyploidisation to occur.

The present chromosomal analysis confirms the conclusion of Laurent (1972) and Tinsley (1973; 1975) that X.vestitus is a distinct species. Although sympatric with X.l.victorinus, X.vestitus differs from the latter by the number of chromosomes and by the morphology of the chromosomes bearing secondary constrictions (TYMOWSKA, submitted to press).

At the present state of our knowledge it would be very difficult to recognize the two ancestral diploid species which may have given rise to X.vestitus. However, some indication can be given of the presence and localization of the nucleolar constrictions. In X.vestitus these constrictions appear terminally on chromosome No.12. If the mutagenic potentiality of nucleolar organizer regions is taken into consideration (TYMOWSKA, submitted to press) an analogy with the nucleolar organizer in subspecies of

X.laevis can be suggested, admitting the possibility of paracentric inversion.

The absolute length of the short arm of pair No.12 reveals similar data for the regions of condensed chromatin and secondary constrictions for this tetraploid species and for Xenopus laevis victorianus in particular.

	secondary constriction	condensed chromatin	total short arm
<u>X.l.laevis</u>	7.27	6.56	13.84
<u>X.l.petersi</u>	7.60	5.47	13.08
<u>X.l.victorianus</u>	8.66	6.16	14.83
<u>X.vestitus</u>	8.45	6.45	14.87

These results could suggest the possibility that the subspecies of laevis and the present tetraploid species have a partially common origin. But further studies are now required on the karyotypes and other characteristics of other Xenopus taxa occurring in Central Africa to attempt to determine the ancestry of this presumably allopolyploid species.

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Running head

TYMOWSKA, FISCHBERG, The hexaploid species
Xenopus ruwenzoriensis

Title

The karyotype of the hexaploid species Xenopus
ruwenzoriensis Fischberg and Kobel (Anura:Pipidae)¹⁾

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ABSTRACT

The chromosomal complement of the hexaploid species Xenopus ruwenzoriensis shows 108 chromosomes which fall usually into hextets of six similar chromosomes which can be classified into the morphological groups characteristic of the genus Xenopus. However, secondary constrictions which appear only on two chromosomes of a particular hextet unmask an underlying heterogeneity. There are three distinct secondary constrictions which are typical for X.ruwenzoriensis only. The secondary constriction demonstrating chromosomal association is considered to be the nucleolar organizer region. There is no evidence for sex-dimorphism amongst chromosomes. Plates of spermatocyte metaphase I often show 54 meiotic figures; however, rare large multivalents have also been observed. In the second spermatocyte metaphase 54 chromosomes are found and X.ruwenzoriensis is, therefore, assumed to be either an ancient autohexaploid, or more likely, an allopolyploid of more modern origin.

INTRODUCTION

Polyploidization, very commonly found in plants, is rather rare amongst animals and particularly in Amphibia. However, in Anura various natural polyploid forms have recently been discovered (BEČAK et al. 1966; BOGART, 1967; BEČAK et al. 1967; BARRIO and RINALDI DE CHIERI, 1970; BEČAK et al. 1970; WASSERMANN, 1970). In the family Pipidae a tetraploid and a hexaploid species of the genus Xenopus have been reported (TYMOWSKA and FISCHBERG, 1973; TYMOWSKA et al., in manuscript). In the present paper the karyotype of the hexaploid species X.ruwenzoriensis is described.

MATERIALS AND METHODS

X.ruwenzoriensis, first found in 1972 by one of us and Dr. H.R. Kobel in the Semliki Valley, Uganda, is a new species (FISCHBERG and KOBEL, in manuscript). Four animals, 2♂♂ and 2♀♀ bred in our laboratory, have been examined cytologically. The technique used is the same as previously described (TYMOWSKA and KOBEL, 1972).

RESULTS AND DISCUSSION

The chromosomal complement of X.ruwenzoriensis is composed of 108 chromosomes (TYMOWSKA and FISCHBERG, 1973). Neglecting the diploid chromosome number of X.tropicalis (TYMOWSKA 1973) which is twenty, and unique in this genus, one may define the basic chromosome number of all other Xenopus species as being $x = 18$. X.ruwenzoriensis would therefore be a hexaploid.

Analysis of the meiotic chromosomes also leads to the same conclusion. At the first meiotic division of the male 54 bivalents are found in most cases (fig.1b), however, there are also cells showing one or more large multivalents (fig.1a). At the second division 54 chromosomes are always observed (fig.1c). A similar situation has been observed at meiosis of the following polyploid species : Odontophrynus americanus (4n) (BEČAK et al.1966), Ceratophrys ornata (8n) (BOGART, 1967), C.dorsata (8n) (BEČAK et al.1967), Phyllomedusa burmeisteri (4n) (BEČAK et al.1970) and Hyla versicolor (4n) (WASSERMANN 1970).

The 108 chromosomes of X.ruwenzoriensis can be fitted into the morphological groups characteristic of the genus Xenopus (TYMOWSKA, submitted to press). Each numbered chromosome is represented by six morphologically similar chromosomes (hextet) instead of the usual pair of homologues (figs. 2,3b,4). However, there are exceptions to this.

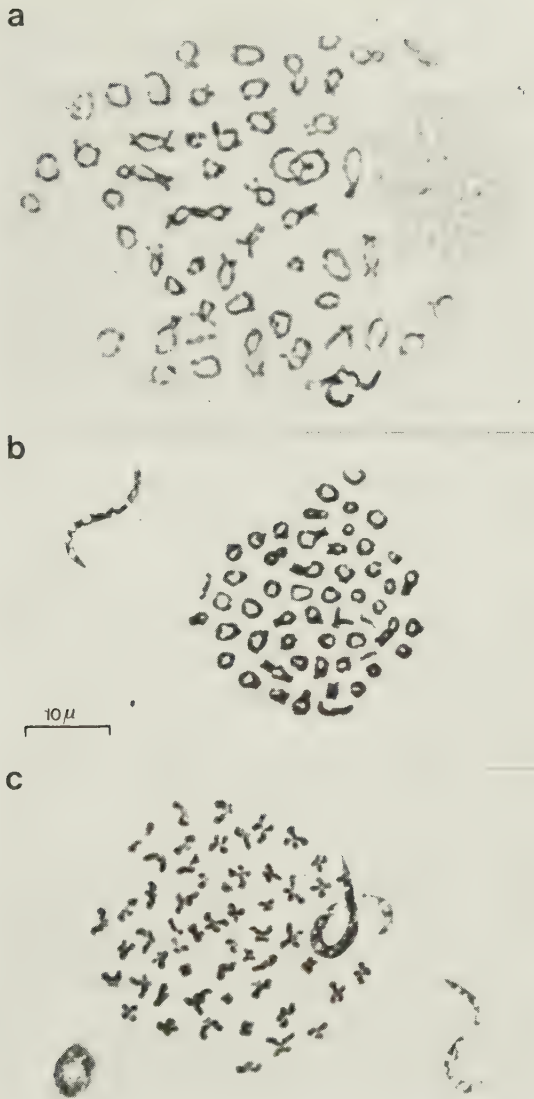


Fig. 1 Male meiosis of *X. ruwenzoriensis*

- a) metaphase I with large multivalents
- b) metaphase I showing 54 meiotic figures
- c) metaphase II showing 54 chromosomes.

In three hextets, possessing chromosomes with secondary constrictions, only two of them show these constrictions, thus exposing a heterogeneity in these hextets. The morphological uniformity of the remaining 15 hextets is probably also only apparent and masks most likely an underlying genetical heterogeneity. This latter would explain the important prevalence of bivalents during male meiosis. Tetraploid species of Xenopus (TYMOWSKA et al. in manuscript) and of Pleurodema (BARRIO and RINALDI DE CHIERI, 1970) also show only two homologues carrying secondary constrictions within the quartets of four chromosomes.

As mentioned above the karyotype of X.ruwenzoriensis can be subdivided into the morphological groups of chromosomes characteristic of the genus Xenopus (see Table I; fig.4). The first group is composed of the three longest submetacentric hextets. The second group contains all medium sized submetacentric chromosomes (Nos. 4 to 7). The hextet No.5 is not homogeneous as only two of six chromosomes possess secondary constrictions. These are localized on the short arms and are attached directly to the centromeres connecting these with the satellites. The third group is formed by a hextet of subacrocentric chromosomes (No.8). The fourth group is also made up by a single hextet of long metacentric chromosomes. Two hextets of small more or less metacentric chromosomes (Nos. 10 and 11) compose the fifth group.

Table I.

Morphometric characteristics of X.ruwenzoriensis chromosomes
(2♂, 2♀; 38 mitoses)

Metastet No	Relative length (o/oo of 6n set)			Arm ratio ($\frac{\text{short}}{\text{long}} \times 100$)		
	Mean	SD	SE	Mean	SD	SE
1	15.62	1.35	0.08	65.86	7.37	0.48
2	13.26	0.89	0.05	55.80	7.73	0.51
3	11.88	0.62	0.04	57.51	7.27	0.48
4	10.35	0.72	0.04	58.91	8.46	0.56
5	9.12	0.60	0.03	70.17	10.07	0.66
6	8.45	0.67	0.04	62.56	11.02	0.73
7	6.99	0.67	0.04	74.13	11.54	0.76
8	10.74	0.94	0.06	41.46	5.96	0.39
9	10.27	0.87	0.05	90.28	7.54	0.49
10	5.88	0.59	0.03	65.38	20.00	1.32
11	4.63	1.05	0.06	76.24	16.26	1.07
12	10.85	0.72	0.04	24.08	6.44	0.42
13	9.81	1.19	0.07	26.41	7.08	0.46
14	9.17	0.39	0.02	24.90	5.12	0.33
15	8.66	1.27	0.08	27.65	6.21	0.41
16	8.13	0.46	0.03	28.65	5.82	0.38
17	7.14	0.74	0.04	34.57	8.03	0.53
18	5.58	1.05	0.06	41.58	9.57	0.63

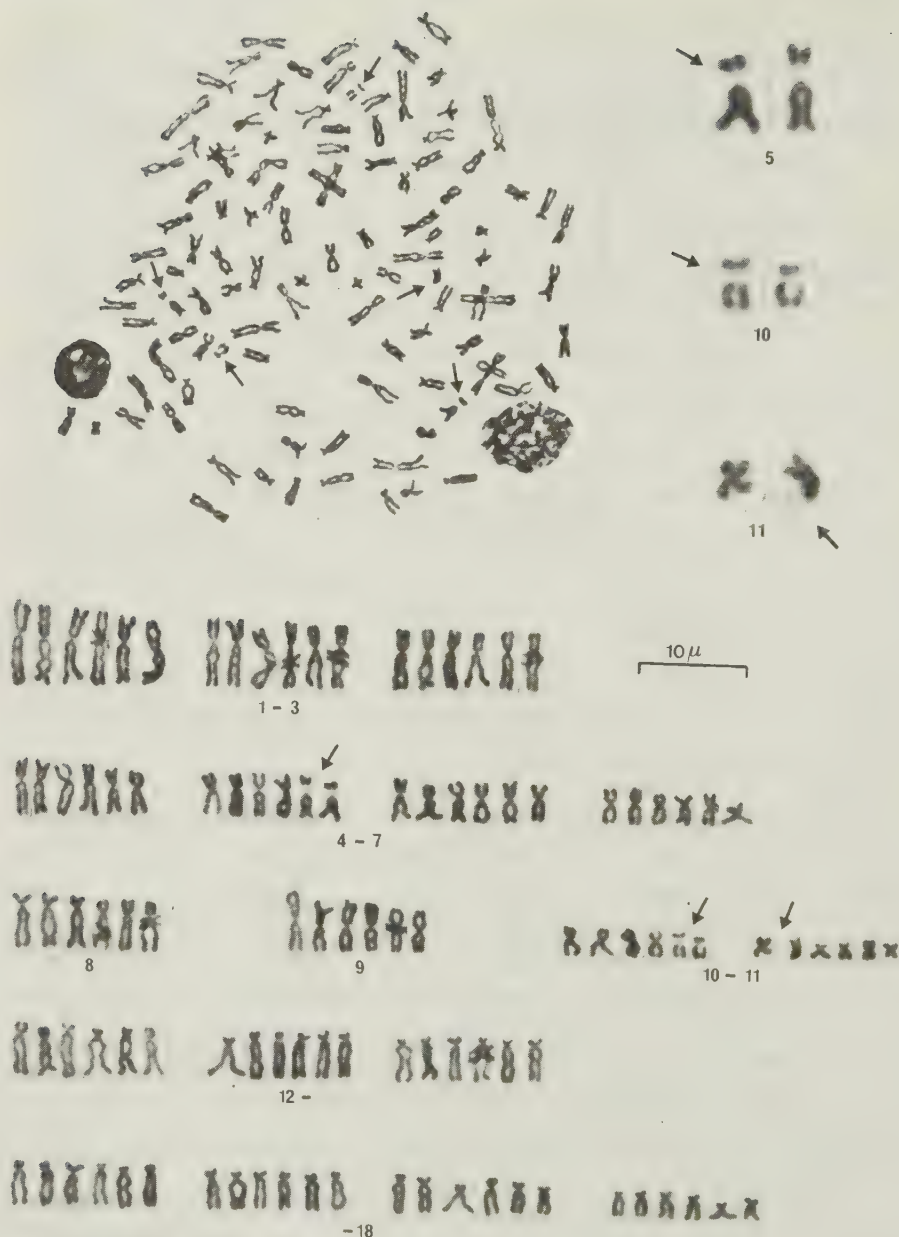


Fig. 2 Mitotic chromosomes and karyotype of *X. ruwenzoriensis*.
Arrows indicate the secondary constrictions.

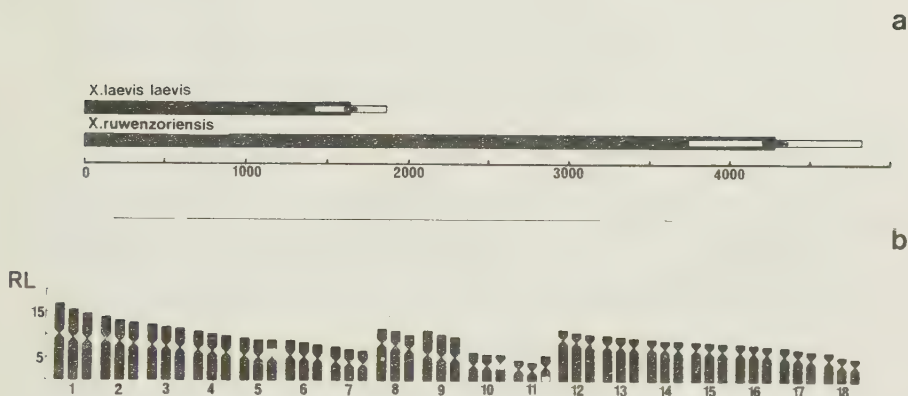


Fig. 3 a) Total length of chromosomal complements of *X.ruwenzoriensis* (mean = 4283.81) as compared to that of *X.laevis laevis* (mean = 1640.83). White rectangles indicate the standard deviation and black ones the standard error of the mean.

b) Idiogram of chromosomes of *X.ruwenzoriensis* based on the measurements of relative length (RL) and arm ratio (AR). The mean length of the two larger, the two middle-sized and the two smallest chromosomes of each hexet was calculated separately.

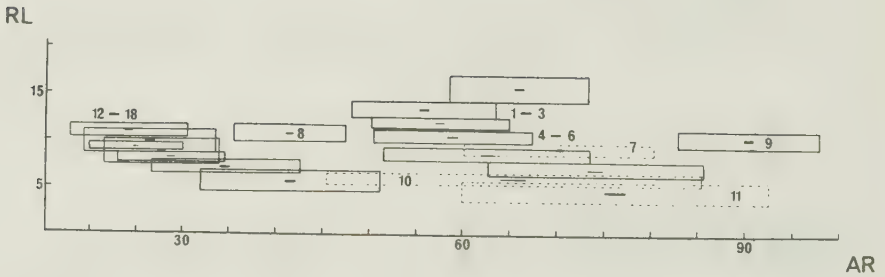


Fig. 4 Two-dimensional karyogram of X. ruwenzoriensis demonstrating the morphological groups of chromosomes based on data of Table I calculated for each of the hextets. Black rectangles indicate the standard error of the mean and white ones the standard deviation. Chromosomes carrying secondary constrictions are designated by dotted lines.

Both are heterogeneous due to the chromosome pairs carrying secondary constrictions. The secondary constrictions of pair No.10, placed within the long arms, are attached on one side to the centromeres and on the other to the long condensed satellites. The secondary constrictions of pair No.11 are terminal and prolongations of the long arms. The last group is composed of seven hexets of acrocentric chromosomes (Nos.12 to 18) arranged in order of size (figs.2, 3b, 4).

The chromosomal pair of hexet No.11, possessing secondary constrictions, shows somatic association in 37% of the analysed mitoses. This association behaviour, previously reported for Xenopus species, is considered to be characteristic of nucleolar organizer constrictions (TYMOWSKA and KOBEL, 1972; TYMOWSKA, submitted to press). By analogy, the secondary constrictions on the two chromosomes of set No.11 are interpreted as nucleolar organizers.

The cytological analysis of X.ruwenzoriensis shows no morphological differences amongst any of its chromosome pairs which could indicate heteromorphism of sex chromosomes. This once again supports the assumption that lower vertebrates have sex-chromosomes which have remained in an undifferentiated state and can facilitate the occurrence of polyploidy (BEÇAK et al. 1970; BOGART and WASSERMANN, 1972).

The total absolute length of the X.ruwenzoriensis chromosomal complement is 161% longer than that of X.laevis laevis (fig.3a). Comparison of the DNA content of these two species reveals similar differences (156% more for X.ruwenzoriensis; THIEBAUD and FISCHBERG, in press). It is, therefore, obvious that X.ruwenzoriensis is a polyploid species.

The meiotic behaviour of the 108 chromosomes of X.ruwenzoriensis and the occurrence of only two chromosomes with secondary constrictions in hextets Nos.5,10 and 11 indicate that diploidization is already far advanced in this hexaploid species. These three secondary constrictions are characteristic of X.ruwenzoriensis and are not found in any other Xenopus taxa, they can, therefore, not help to shed more light on the origin of this species. None the less, a hybrid origin, having lead to allopolyploidy, is suggested as this would facilitate bivalent formation as opposed to multivalent formation in meiosis. A likely mechanism for polyploidization in Xenopus hybrids has already been discovered by Muller (submitted to press) and Kobel and Du Pasquier (1975). These authors found a variable percentage of tetraploid oocytes in different diploid species hybrids of the genus Xenopus, oocytes which, after meiosis, develop into viable diploid eggs. Such eggs fertilized by a haploid non-hybrid sperm give rise to triploid frogs.

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Running head

TYMOWSKA, FISCHBERG, The tetraploid Xenopus sp.n.

Title

The karyotype of Xenopus sp.n. Fischberg, Tinsley
and Kobel, another tetraploid anuran species (Pipidae) ¹⁾

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NOTE

The publication of this manuscript, concerning Xenopus species nova will be held back until M. Fischberg, Drs. R.C. Tinsley and H.R. Kobel, who found this new species, will have named it and will have published its description.

ABSTRACT

The analysis of the karyotype of Xenopus sp.n. brought another case of tetraploidy among Anura to light. The chromosome number is 72. During first male meiosis 36 bivalents are found and during second male meiosis 36 chromosomes can be seen. Instead of the usual pairs of homologues, quartets of four similar chromosomes are found which fall into the same basic morphological groups which are typical for the genus Xenopus. The secondary constrictions on chromosome No.12 show somatic association and are, therefore, considered to be the nucleolar organizers. They appear only on two homologous chromosomes of the quartet. It is likely that X.sp.n. represents a case of allotetraploidy.

INTRODUCTION

The number of reported polyploid species amongst Anura increases rapidly. Polyploidy is now considered to represent an important evolutionary mechanism for some of the genera (BOGART and WASSERMANN, 1972).

Recently three polyploid species have been discovered in the genus Xenopus; a hexaploid species, X.ruwenzoriensis Fischberg and Kobel (in manuscript) which possesses 108 chromosomes (TYMOWSKA and FISCHBERG, 1973), a tetraploid species, X.vestitus Laurent (1972; TINSLEY 1973, 1975; TYMOWSKA, FISCHBERG and TINSLEY, in manuscript) with 72 chromosomes and finally X.sp.n. Fischberg, Tinsley and Kobel (in preparation) the tetraploid species of which the karyotype is being described in this paper.

MATERIAL AND METHODS

Four live specimens of Xenopus sp.n., found in 1972 by one of us (M.F.) and Dr. H.R. Kobel in Lake Bunyoni and in an artificial fishpond near Kashasha (Uganda) have been analysed in detail; two ♀♀ and two ♂♂. Five more animals (2 ♀♀ and 3 ♂♂) from two other populations also in the Kigezi district in western Uganda (Chelima Forest and Echuya) were used for confirmation of the chromosome number and chromosome morphology. (These 5 animals were found in 1975 by M.F., Dr.R.C. Tinsley, Dr.H.R. Kobel and Dr.L. Du Pasquier). The chromosome preparations were made by the dry-air method and ten karyotypes of each of the 4 animals were analysed in detail as previously described (TYMOWSKA and KOBEL, 1972).

RESULTS AND DISCUSSION

The chromosome number of all 9 analysed individuals of Xenopus sp.n. is 72, the same as was recently reported for another species, X. vestitus (TYMOWSKA, et al., in manuscript). This chromosome number corresponds to tetraploidy (figs. 2,3).

The pairing behaviour of the chromosomes during male meiosis is similar to that normally found in a diploid organism. The first meiotic metaphase possesses 36 bivalents while the second meiotic metaphase contains 36 chromosomes (figs. 1a,b). A similar situation is found in X. vestitus and other tetraploid anuran species of the genus Pleurodema (BARRIO and RINALDI DE CHIERI, 1970).

The 72 chromosomes of X.sp.n., arranged according to size and centromere position, form the same morphological chromosome groups (Table I, fig. 4) which are characteristic of the Xenopus species possessing 36 chromosomes (TYMOWSKA, submitted to press). The fact that each chromosome is represented by a quartet of four similar chromosomes instead of the usual pair of two homologues as in diploid species, also indicates tetraploidy. However, chromosomal quartets including chromosomes bearing secondary constrictions reveal a more or less hidden heterogeneity as there are only two homologues with secondary constrictions per quartet. This is also observed in the genus Pleurodema (BARRIO and RINALDI DE CHIERI, 1970).

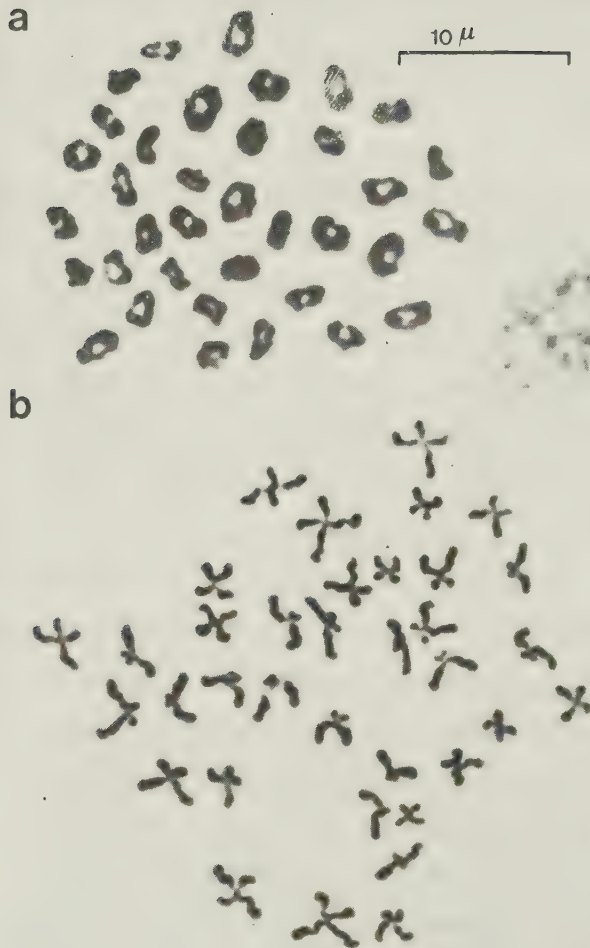


Fig. 1 Male meiosis of *X.sp.n.*
a) First metaphase showing 36 bivalents
b) Second metaphase showing 36 chromosomes.

The karyotype of X.sp.n. is composed of several groups of chromosomes (figs. 2-4). The three longest submetacentric quartets (Nos. 1 to 3) form the first morphological group. The second group is composed of the medium-sized submetacentric chromosomes (Nos. 4 to 7). The third group contains only one quartet (No 8), which is subacrocentric. The large metacentric chromosomes (No.9) form the next group. The fifth group is made up by two quartets of small metacentric chromosomes (Nos. 10 and 11). The longest acrocentric quartet (No.12) is heterogeneous. Two of the four chromosomes possess secondary constrictions which are placed terminally on the short arms. The last group contains all the remaining acrocentric chromosomes (Nos.13-18) (figs. 2,3,4).

The two homologous chromosomes of No.12 which bear secondary constrictions show somatic associations in 23,4% of the 115 mitoses examined. This particular behaviour is considered to involve the nucleolar organizer regions (TYMOWSKA and KOBEL, 1972; TYMOWSKA, submitted to press). Therefore, the constrictions on chromosome pair No. 12 of X.sp.n. are thought to be the nucleolar organizers.

The cytological analysis of X.sp.n. does not reveal any morphological differences among chromosomes which might be interpreted as chromosomal sex-dimorphism. Since this situation is found in all analysed Xenopus species (MIKAMO and WITSCHI, 1966; GEHRING and HAUSCHTECK-JUNGEN, 1967;

Table I.

Morphometric characteristics of the chromosomes
of Xenopus sp.n. (2 ♂♂, 2 ♀♀; 38 mitoses)

Quartet No.	Relative length (o/oo of 4n set)			Arm ratio($\frac{\text{short}}{\text{long}}$ x 100)		
	Mean	SD	SE	Mean	SD	SE
1	22.20	3.40	0.27	67.68	8.08	0.65
2	19.07	2.64	0.21	58.13	8.32	0.67
3	17.50	2.34	0.19	57.30	6.34	0.51
4	16.24	2.10	0.17	56.38	8.11	0.65
5	14.33	2.22	0.18	58.89	9.56	0.77
6	12.52	1.50	0.12	64.48	10.51	0.85
7	11.67	1.46	0.11	61.68	10.36	0.84
8	15.63	1.92	0.15	39.27	5.98	0.48
9	15.27	2.12	0.17	89.41	7.96	0.64
10	10.69	1.45	0.11	77.04	10.73	0.87
11	9.35	1.23	0.10	72.72	12.55	1.01
12	15.95	2.74	0.22	29.35	9.59	0.77
13	14.36	2.06	0.16	29.45	5.80	0.47
14	13.33	1.85	0.15	27.41	5.25	0.42
15	12.69	1.61	0.13	29.89	5.86	0.47
16	12.19	1.46	0.11	30.89	6.95	0.56
17	11.53	1.38	0.11	31.31	5.39	0.43
18	10.56	1.36	0.11	36.02	5.81	0.47

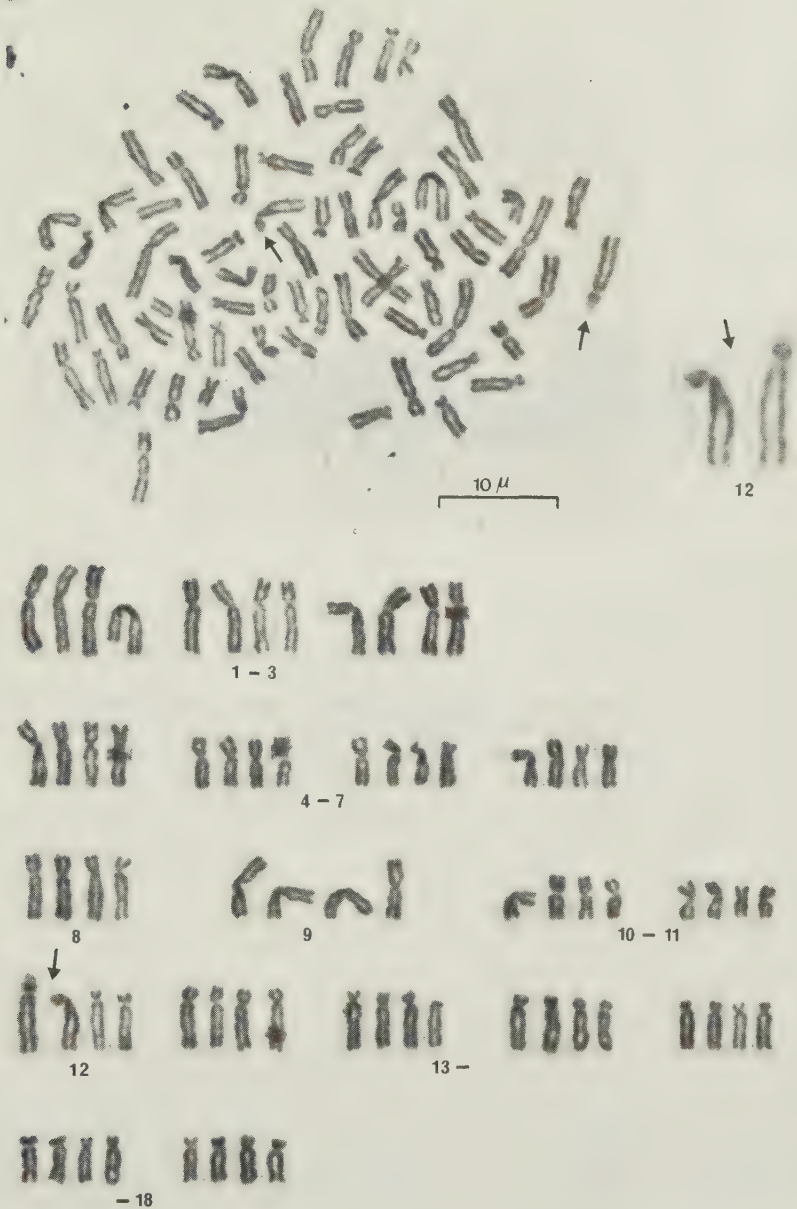


Fig. 2 Mitotic chromosomes and karyotype of *X.sp.n.*
The arrows indicate secondary constrictions.



Fig. 3 Idiogram of *X.sp.n.* based on the measurements of relative length (RL) and arm ratio (AR); see also Table I.



Fig. 4 Two-dimensional karyogram of *X.sp.n.* showing distribution of chromosomal groups, based on measurements of RL and AR (Table I). Black rectangles indicate the standard error of the mean and white ones the standard deviation. Dotted lines designate the chromosomes with secondary constrictions.

TYMOWSKA and KOBEL, 1972; TYMOWSKA, 1973; TYMOWSKA and FISCHBERG, 1973; TYMOWSKA et al., in manuscript) it can be assumed that the sex-chromosomes of these animals remain in a relatively undifferentiated state which might facilitate polyploidization.

In comparison to X.laevis laevis (100%) the total absolute length (mean) of the 72 chromosomes of X.sp.n. is almost doubled (182.7%). This increased length is not due to some exceptionally large chromosomes, but to the doubled chromosome number (fig. 6). In fact the chromosomes of X.sp.n. are usually slightly shorter than those of X.l.laevis. This is probably not due to DNA-losses but rather to a stronger condensation of the X.sp.n. chromosomes. Thiébaud and Fischberg (submitted to press) showed that the DNA content of X.sp.n. per nucleus is exactly twice that of X.l.laevis (198% : 100% or 12.57 pg/nucleus : 6.35 pg/nucleus). It is, therefore, concluded that X.sp.n. represents, like X.vestitus, a case of tetraploidy and probably one of allotetraploidy. Allotetraploidy is suggested on grounds of the meiotic behaviour of the chromosomes and the relatively frequent occurrence of the tetraploid oocytes in Xenopus species hybrids (KOBEL and DU PASQUIER; 1975).

The karyotypes of these two tetraploid Xenopus species are very similar to one another. The only differences being the presence of a constriction on chromosome No. 2

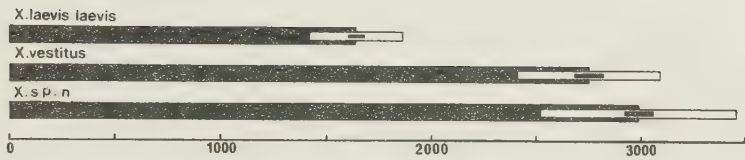


Fig. 5 Total absolute length of chromosome complements of *X.l.laevis* (mean = 1601.83), *X.vestitus* (mean = 2756.25) and *X.sp.n.* (mean = 2999.42). White rectangles indicate the standard deviation and black ones the standard error of the mean.

in X.vestitus and the shorter length of all X.vestitus chromosomes which is expressed in the slighter total absolute length (figs. 5 and 6). The terminal secondary constriction on chromosome No. 12 is present in both species and is interpreted to represent the nucleolar organizer. The similarity of the karyotypes of the two species suggests that they have at least one ancestor in common.

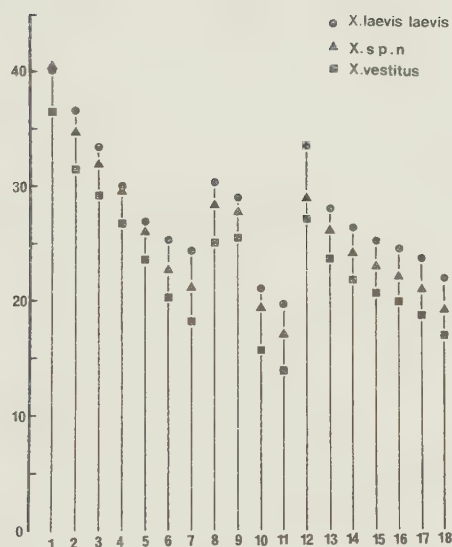


Fig. 6 Comparison between the chromosomal length of *X.l.laevis*, *X.sp.n.* and *X.vestitus*. The idiogram is made by multiplying the relative length of each chromosome of the two tetraploid species by a factor corresponding to the difference in total absolute length as compared to *X.l.laevis* (i.e. for *X.sp.n.* $\times 1.82$ and for *X.vestitus* $\times 1.67$; see also TYMOWSKA and KOBEL, 1972 and TYMOWSKA et al., in manuscript).

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GENERAL DISCUSSION

The chromosome numbers within the order Anura vary generally little, maintaining thus a constancy within the large genera and even within the families. Some families, even those which possess numerous species, such as Bufonidae ($2n = 22$ and $2n = 30$), Hylidae ($2n = 24$, 22 , 30), Ranidae ($2n = 26$) and Leptodactylidae ($2n = 26$, 22) have remarkably homogeneous chromosome numbers (BLAIR, 1972; MORESCALCHI, 1973).

However, the genus Xenopus shows great heterogeneity of the chromosome number amongst its species. The majority of species has a diploid number of 36. One species, X.tropicalis, possesses $2n = 20$; two species, X.sp.n. and X.vestitus have a chromosome number of 72, and one species, X.ruwenzoriensis, is characterized by a still higher number (108) of chromosomes (TYMOWSKA and FISCHBERG, 1973).

Cytological analysis including the measurement of the total length of the whole chromosome complements, clearly demonstrates that in the cases of the species X.sp.n., X.vestitus and X.ruwenzoriensis we are dealing with the phenomenon of polyploidy. Further, measurements of DNA values entirely confirm this conclusion (THIÉBAUD and FISCHBERG, in press). The two species with a 72 chromosome complement are tetraploid ($4n$) and the species with 108 chromosomes is

hexaploid ($6n$). The basic chromosome number of this genus seems to be $x = 18$.

The karyotypes of the Xenopus species analysed, with the exception of X.tropicalis, are very uniform in terms of size and morphology of the chromosomes. The haploid karyotype shows 18 chromosomes which can be divided into two principal groups of submetacentric and acrocentric chromosomes. Morescalchi (1968) considers such karyotypes, formed by large chromosomes, to be characteristic of higher Anura. However, the karyotype of Xenopus is unique amongst existing Anura and there are even no morphological similarities between Xenopus and the other Pipids (see also MORESCALCHI, 1973).

The mechanism of fusion or fission regarded as a frequent event in speciation of many anurans (MORESCALCHI, 1973; BOGART, 1970, 1973; SCHEEL, 1971) could not be detected in Xenopus due to the technique used and the similarity of their karyotypes (with the exception of X.tropicalis), moreover no telocentric chromosomes were observed.

The lack or the very low frequency of bivalents observed in meiosis of interspecific hybrids as compared to the normal pairing in oocytes of intraspecific hybrids (Müller, unpublished) indicate that the morphologically similar chromosomes of various Xenopus species differ greatly in genetic structure. This means that many single or multiple changes at the gene level, involving small amounts of chromatin, might have arisen during speciation.

This does of course not exclude the possibility of inversions and translocations having occurred as well. The existence of small differences in the genome length amongst the species examined, probably indicates variable amounts of heterochromatin, although no study concerning heterochromatin has been undertaken. In the case of some species the increased genome length is more likely due to differences in the degree of chromatin condensation.

The only obvious structural diversity of Xenopus karyotypes is the morphology, position and number of secondary constrictions. The same situation has been found for the genus Bufo (BOGART, 1972) in which all species possessing 22 chromosomes have uniform karyotypes; only due to the secondary constrictions can some be distinguished from others.

The secondary constrictions of Xenopus are in the majority of cases nucleolar organizer regions or are at least suspected to be such. There is in general one pair of chromosomes, possessing a secondary constriction which shows somatic association at a frequency of about 25-50%. The phenomenon of somatic chromosome association is in most cases our criterium to determine which of the constrictions are nucleolar organizers and are, therefore, homologous to the respective regions of other species. Measurements of the length of these constrictions show that they vary very little between species including polyploid ones.

There are, however, two exceptions: the nucleolar constrictions of X.fraseri and the two types of secondary constrictions showing association behaviour in X.tropicalis. In both species the constrictions are only half the length of those of other Xenopus species.

The comparison of Xenopus karyotypes reveals that nucleolar organizers can appear on different chromosomes and in different positions within the chromosome (interstitially, proximally to the centromere or distally). It can, therefore, be assumed that these regions manifest a high mutability and possess a strong evolutionary potentiality. The well-known Oxford nucleolar marker mutation (ELSDALE et al., 1958), a deletion, (KAHN, 1962), the mutation in X.borealis which is due to an additional nucleolar constriction, probably resulting from a reciprocal translocation, (JOTTERAND and FISCHBERG, 1974) and the nucleolar mutation in X.fraseri described above are examples of the mutability of this region.

It is of course possible that the nucleolar organizer is not really more labile than other parts of the chromosomes. The secondary constriction is easily recognizable and any change in its number or position does not escape the observer. Deletions, translocations or inversions affecting other parts of chromosomes would not show up clearly, unless the rather laborious banding technique

would be used. Should chromosomal rearrangements have played an important role in the evolution of the genus Xenopus, its rather conservative karyotype would be difficult to explain.

Attempts to clarify the relationship between Xenopus-species on the basis of our cytological criteria are limited to a comparison of the position of secondary constrictions. Nevertheless, the close relation between the three subspecies X.l.laevis, X.l.victorianus and X.l.petersi could be confirmed; the cytological similarity of X.borealis to X.muelleri and the dissimilarity to the X.laevis group could also be demonstrated (TYMOWSKA and FISCHBERG, 1973). X.gilli can be interpreted as an intermediate between muelleri and laevis. In the case of X.clivii, assuming there are paracentric inversions involving the chromosomes with secondary constrictions, the possible proximity of this species to the muelleri type may be considered. X.fraseri possessing a particular nucleolar constriction, characteristic for this species only, seems rather distant to the remaining Xenopus taxa. The two tetraploid species X.vestitus and X.sp.n. having similar nucleolar organizer constrictions, might be closely related. The terminal location of these secondary constrictions might be the result of an inversion within the short arm of the satellite chromosome No 12 which might originally have resembled that of the X.laevis group. Should this have been the case it would indicate a common origin of the present

subspecies of laevis with the tetraploid species X.vestitus and X.sp.n. Measurements of the short arm of pair No 12 gave similar data for the region of condensed chromatin and the constriction itself for both, the tetraploid species and also all the subspecies of Xenopus laevis.

Measurements of the short arm of chromosome No. 12

	Secondary constriction	Condensed chromatin	Total length
X.l.laevis	7.27	6.56	13.8
X.l.petersi	7.60	5.47	13.08
X.l.victorianus	8.66	6.16	14.83
X.vestitus	8.45	6.45	14.87
X.sp.n.	6.73	8.41	15.14

These data do not prove a common origin of laevis subspecies with the two tetraploid species. However, they do not exclude this possibility. The nucleolar organizer region of the hexaploid species X.ruwenzoriensis is typical for this only and at present no particular relationship with any of the other Xenopus species can be deduced from the cytological data in hand.

During recent years several taxa of naturally occurring bisexual anuran polyploids have been reported within the families of Ceratophrydidae, Leptodactylidae and Hylidae (M.L. BEÇAK et al., 1966; BOGART, 1967; BEÇAK et al., 1967; BARRIO and RINALDI DE CHIERI, 1970a;

BEÇAK et al., 1970; WASSERMAN, 1970). Five of these species are tetraploid (Odontophrynus americanus, Pleurodema bibroni, P.krieqi, Phyllomedusa burmeisteri, Hyla versicolor) and two are octoploid (Ceratophrys ornata and C.dorsata). With the exception of the Pleurodema species all show multivalents at meiosis and are thus interpreted as auto-tetraploid. Moreover, for the polyploid forms Odontophrynus americanus, Ceratophrys ornata, Phyllomedusa burmeisteri and Hyla versicolor (BOGART and WASSERMAN, 1972; BARRIO and RINALDI DE CHERI 1970b; BATISTIC et al., 1975) there exist similar diploid counterparts in nature. Bogart (1972) designated the above species as "diploid-polyploid cryptic species pairs". The existence of these similar diploid populations also indicates autopolyploidy and at the same time suggests a recent and sudden process of polyploidization. There has not been sufficient time for diversification, the formation of an intermediate population, or the elimination of the diploid populations to occur. This polyploidization seems to be a unique process of speciation, established potentially in one generation, contrary to speciation by isolation which proceeds slowly and gradually through many generations (BATISTIC et al., 1975).

The situation concerning the polyploid species of Xenopus is different. Amongst the diploid Xenopus species so far examined no diploid types corresponding to the polyploid species have been found. Xenopus polyploids are also in general different from other polyploid anurans by

the regular occurrence of bivalents during meiosis. Polyploid Pleurodema species only resemble those of Xenopus in these two aspects. It seems, therefore, that the mechanism of polyploidization for these two genera proceeded in another way. There is strong evidence to consider the polyploidy found in Pleurodema and Xenopus as allopolyploidy, established long enough to bring about diploidization. Allopolyploidy occurs by duplication or multiplication of the hybrid genomes of two species, perhaps in order to counteract chromosomal imbalance leading to sterility or lethality due to irregular segregation during meiosis. From hybridization experiments with Bufo (BLAIR, 1972) it is known that polyploid hybrids are more vigorous than diploid hybrids. By analogy spontaneous Xenopus species hybrids would stand a better chance of survival and maintenance in nature after polyploidization. The occurrence of tetraploid oocytes in Xenopus hybrids, obtained in our laboratory, clearly suggests the possibility of such a mechanism (MÜLLER, unpublished; KOBEL and DU PASQUIER, 1975). Polyploid Xenopus hybrid oocytes result from a supplementary duplication of chromosomes (MÜLLER, unpublished). A corresponding duplication in the testis of males could theoretically lead to the formation of $2n$ sperms and so to the creation of a new allotetraploid species.

Hybridization between Xenopus species, carried out in the laboratory, leads to partially fertile females and generally to total sterility in males (VIGNY, 1976).

It is, therefore, not likely that the diploid eggs, resulting from tetraploid oocytes are fertilized by diploid hybrid sperm. Much more likely is the fertilization of diploid hybrid eggs by haploid sperm of one of the parental species, resulting in a triploid hybrid of type AAB (VIGNY, 1976). Such $3n$ hybrids can produce hexaploid oocytes, due to another supplementary duplication of chromosomes (MOLLER, 1976), and after meiosis to triploid eggs. Triploid eggs fertilized by haploid sperm give rise to tetraploid hybrid frogs, which can be of the type AABB and, therefore, genetically more or less balanced. Hybridization can thus almost instantly provoke a doubling of the allo genomes which would permit regular meiotic segregation, a necessary condition for the establishment of a new species. Furthermore, structural rearrangements could lead to diploidization, as observed by Kobel for polyploid Xenopus species (unpublished).

It has been found that the amount of DNA in the hexaploid species X.ruwenzoriensis is smaller (2.56) than the expected tripled value of the DNA content of diploid cells (THIEBAUD and FISCHBERG, in press). Similar results are obtained by comparing the total chromosomal length of polyploids and diploids. Like diploid species, the polyploid Xenopus species possess only one pair of homologous chromosomes with nucleolar organizers. In tetraploid and hexaploid species two or three pairs, respectively, of nucleolar regions would be expected. It can, therefore,

be assumed that during speciation a reduction of these redundant genetic loci has occurred and a diploid condition has been attained.

Since in all Xenopus species analysed, sex-chromosomal dimorphism could not be observed, one may infer that in these lower vertebrates sex-chromosomes are still in a rather primitive stage. This ought to facilitate the occurrence and fixation of polyploidy.

The small number of chromosomes ($2n = 20$) found in X.tropicalis amounts to nearly half of the 36 chromosome complements of the majority of other Xenopus species. This implies that all species bearing 36 chromosomes can be considered as polyploids (tetraploids) in respect to X.tropicalis. If one accepts that the process of polyploidization occurred in the past, distant enough to have allowed these species to attain a genetic equilibrium through diploidization, then one can assume the existence of a common ancestor for X.tropicalis and all the other species. This common ancestor would have possessed a chromosomal number of $2n = 18$ or 20 . This being probably the case, the chromosomal number of the two tetraploid species X.vestitus and X.sp.n. would in reality correspond to octoploidy and the chromosome number of X.ruwenzoriensis to dodecaploidy.

It is worth mentioning that Hymenochirus boethgeri of another African genus of the family Pipidae has a chromosome number of $2n = 24$ (MORESCALCHI, 1968a) and that its karyotype resembles, to a certain extent, the karyotype of X.tropicalis.

A recent paleontological study reports a fossil Xenopus, X.romeri, in Brazil from the Paleocene (ESTES, 1975). A strong resemblance exists not only between this fossil and living X.tropicalis but also with fossils of X.tropicalis found in Morocco from the Miocene. These findings indicate that X.tropicalis is a very ancient, well-established species, and that sufficient time has passed for polyploidy to arise and to be fixed in this genus.

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RESUME

Ce travail a permis d'établir et de comparer les caryotypes des espèces et sous-espèces de Xenopus dont je disposais. 12 espèces et sous-espèces de Xenopus ont été analysées : Xenopus laevis laevis (Daudin), X.l.petersi Bocage, X.l.victorianus Ahl, X.borealis (Parker), X.gilli Rose et Hewitt, X.muelleri (Peters), X.clivii Peracca, X.tropicalis (Gray), X.fraseri Boulenger, X.vestitus Laurent, X.sp.n. Fischberg, Tinsley and Kobel, X.ruwenzoriensis Fischberg and Kobel.

-Nombre chromosomique

La majorité des espèces et sous-espèces possède un nombre chromosomique ($2n$) de 36; seul Xenopus tropicalis a un nombre diploïde plus petit, $2n = 20$. 3 espèces sont des polyploïdes naturels : 2 tétraploïdes, X.vestitus et X.sp.n., $4n = 72$, et une hexaploïde, X.ruwenzoriensis, $6n = 108$.

-Longueur totale absolue du caryotype

Les mesures montrent que la longueur totale des caryotypes à 36 chromosomes est presque égale. Ceux de X.muelleri et X.borealis sont les plus longs.

Chez X.tropicalis, cette longueur est environ la moitié de celle mesurée chez X.laevis, ce qui démontre que les chromosomes de X.tropicalis ne sont pas plus grands que ceux des autres espèces.

Les caryotypes tétraploïdes sont presque 2 fois plus longs que celui de laevis. Si, chez ce dernier, cette longueur est égale à 100, elle est de 167 chez X.vestitus, de 182 chez X.sp.nov., enfin de 260 chez l'hexaploïde X.ruwenzoriensis. Ces données correspondent assez bien aux mesures de DNA effectuées sur des érythrocytes par Thiébaud et Fischberg.

-Les groupes chromosomiques

Les mesures de la longueur relative des chromosomes et le rapport de longueur entre les bras chromosomiques ont permis d'établir des groupes morphologiques des chromosomes et des idiogrammes pour chaque espèce et sous-espèce. Comme ceux-ci sont très semblables, il a été possible de construire finalement un idiogramme hypothétique du genre Xenopus.

Dans un caryotype, on reconnaît 6 groupes chromosomiques distincts.

paire	de chromosomes No.1 - 3 : grands submétacentriques;
paire	No.4 - 7 : petits submétacentriques;
paire	No.8 : subacrocentriques;
paire	No.9 : grands métacentriques;
paire	No.10-11 : petits métacentriques;
paire	No.12-18 : acrocentriques;

On retrouve ce même groupement dans tous les caryotypes des espèces possédant 36 chromosomes également chez les espèces polyploïdes. X.tropicalis est un cas particulier.

-Constrictions secondaires

La principale différence qui existe entre les caryotypes des différentes espèces est le nombre et la position des constrictions secondaires. Les chromosomes qui portent les constrictions secondaires forment un groupe en eux-mêmes.

-Organisateur nucléolaire

Certaines constrictions secondaires sont reconnues comme organisateurs nucléolaires. En 1962, Kahn a trouvé que la constriction secondaire du chromosome porteur de satellite chez Xenopus laevis constitue l'organisateur nucléolaire. Cette conclusion a été confirmée par Pardue (1973) au moyen de la méthode d'hybridation "in situ".

En analysant les chromosomes de X.laevis, on observe que 2 chromosomes homologues possédant l'organisateur nucléolaire présentent, dans 50% des cas, une association mutuelle. On interprète cette association comme le résultat d'une association antérieure des organisateurs à deux nucléoles fusionnés en un seul. Chez toutes les autres espèces de Xenopus, on retrouve le même phénomène, ce qui nous permet de déterminer pour chaque espèce à quelle constriction secondaire correspond l'organisateur nucléolaire.

-Comparaison des caryotypes.

1. Espèces à 36 chromosomes

En se basant sur la comparaison des caryotypes, on peut essayer d'établir le degré de parenté systématique entre les espèces. Les 3 sous-espèces X.l.laevis, X.l.petersi et X.l.victorianus possèdent le même caryotype avec la constriction secondaire située sur le chromosome porteur de satellite No.12, ce qui confirme leur appartenance à une même espèce.

X.borealis possède 2 paires distinctes de chromosomes avec des constriction secondaires qui sont différentes de celles de X.laevis, mais semblables à celles de X.muelleri. X.borealis est maintenant considérée

comme une espèce distincte proche de X.muelleri, et non plus comme une sous-espèce de X.laevis. Le caryotype de X.gilli peut être considéré comme intermédiaire car il présente 2 paires de constriction, l'une de type X.laevis, l'autre de type X.muelleri.

Chez X.clivii, si l'on admet qu'une inversion paracentrique a porté sur le bras court du chromosome No.4 où se trouve la constriction secondaire, on peut établir une homologie avec la constriction terminale du même chromosome chez X.muelleri.

X.fraseri semble plutôt éloigné des autres espèces de Xenopus, son caryotype présentant une constriction nucléolaire particulière à cette espèce.

2. Espèces polyploïdes

Les caryotypes des 2 espèces tétraploïdes se ressemblent beaucoup; on retrouve les mêmes groupes morphologiques avec 4 chromosomes semblables au lieu de 2 homologues. Le chromosome No.12 fait une exception car 2 des 4 chromosomes semblables seulement portent les constriction secondaires. Ces constriction, qui montrent 39% d'association chez X.vestitus et 23.4% chez X.sp.n., sont considérées comme étant les organisateurs nucléolaires. Xenopus vestitus possède encore une autre constriction secondaire ce qui constitue la seule diffé-

rence nette entre les 2 caryotypes tétraploïdes.

Chez ces espèces, à la première division méiotique, on observe seulement 36 bivalents et pas de multivalents; à la 2ème division, on ne compte que 36 dyades; ceci permet de conclure à un cas d'allotétraploïdie.

Le caryotype hexaploïde de X.ruwenzoriensis présente également les mêmes groupes de chromosomes avec des hextets de 6 chromosomes semblables. Les 3 différentes constriction secondaires apparaissent seulement sur 2 chromosomes homologues des hextets. Une seule est considérée comme organisateur nucléolaire car elle montre le phénomène d'association. Pendant la première division méiotique, on retrouve une majorité de bivalents mais aussi parfois quelques grands multivalents; à la 2ème division, on observe 54 chromosomes.

3. Xenopus tropicalis

Le caryotype de cette espèce diploïde à 20 chromosomes est totalement différent de ceux des autres espèces. Il y a 2 constriction secondaires différentes pouvant s'associer. X.tropicalis est considérée comme une espèce systématiquement assez éloignée des autres Xenopus.

-Dimorphisme sexuel

Contrairement aux conclusions de Weiller et Ohno (1962), l'analyse cytogénétique de toutes ces espèces n'a pas montré de chromosomes sexuels morphologiquement reconnaissables. L'existence d'espèces polyploïdes plaide en faveur de l'état relativement peu différencié des chromosomes sexuels chez Xenopus.

CONCLUSION

Cette analyse permet un certain nombre de conclusions :

- 1) Les nombres diploïdes peuvent être très variés chez les anoures, même à l'intérieur d'un genre relativement petit, comme Xenopus.
- 2) La présence des constriction secondaires est une caractéristique importante de chaque caryotype et peut être utile comme critère de parenté systématique entre les espèces.
- 3) Le phénomène d'association est en rapport direct avec la fonction de la constriction secondaire, donc de l'organisateur nucléolaire.
- 4) La potentialité mutagène de la région de l'organisa-

teur nucléolaire est élevée.

- 5) L'existence d'espèces polyploïdes naturelles indique que la polyploïdisation a joué un rôle important dans l'évolution du genre Xenopus.

